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Improvement of varietal aroma in grape and tropical fruit wines by optimal choice of yeasts and nutrient supplements

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ABSTRACT

The main objective of this study was to improve fermentation of yellow passion fruit (YPF) juice by an adequate choice of commercial *Saccharomyces* yeast strains and nutrient supplements.

Comprehensive information has been gathered on the basis of the experimental results obtained from the research on grape wines of the varieties Scheurebe and Sauvignon blanc. The study showed that under laboratory conditions, the optimal commercial yeast strains for the fermentation of Scheurebe wine are strains VIN13, AWRI R2, EC1118, QA23, VL3, X5 and X16 and the most effective nutrients are diammonium hydrogen phosphate (DAP), Fermaid E blanc (DAP, thiamine, yeast cell walls and ammonium sulphate) and Superstart (inactivated yeast and yeast cell walls) at 0.3 g L^{-1}. Yeast strains X5, Alchemy I and II, EC1118 and VL3 and/or the nutrient sources of 0.4 g L^{-1} Fermaid E blanc, 0.5 g L^{-1} DAP and 0.3 g L^{-1} Superstart seemed to be the most effective for the fermentation of Sauvignon blanc wine.

The optimal choice of commercial *Saccharomyces* yeast strains and nutrient supplementations in YPF juice was studied in detail based on the results, which were obtained from grape wines.

YPF puree was used for fermentation trials. The puree has a high acidity (4.0-5.5 % citric acid). Therefore, it was diluted by the addition of water, thus this prepared YPF juice showed a lower amino nitrogen level. Consequently, there is a need for nutrient supplementation, because nitrogen deficiencies are linked to slow and sluggish fermentations and the formation of SO_2-binding compounds and reduced sulphur compounds.

The prepared YPF juices were fermented at 20 ^{0}C under controlled conditions and the fermentation characteristics, fermentation-derived and varietal aromas which impact YPF wine quality were investigated. In order to select adequate commercial yeast strains for the improvement of YPF wine, five *Saccharomyces cerevisiae* var. *bayanus* strains (EC1118, AWRI R2, LittoLevure, QA23 and Freddo), seven *Saccharomyces cerevisiae* strains (Sauvignon, VL3, X5, X16, VIN13, 4F9 and LVCB) and two *Saccharomyces spp.* strains (Alchemy I and II) were examined. The results revealed that when 0.5 g L^{-1} DAP was added as sole source of nitrogen in the YPF juice, strains QA23, LittoLevure, EC1118, X5, VL3 and Alchemy I and II seemed to be an optimal choice for the YPF wine production.

Yeast strains EC1118, VL3, X5, Sauvignon, Alchemy I and LittoLevure were then selected to study the yeast fermentation behaviour and the production of metabolic compounds as well as the volatile thiols in YPF wines. In addition, different nutrient supplements were tested to select an appropriate nitrogen source and concentration. The results clearly showed that nutrient supplements like Vitamon Combi (DAP and thiamine) and Vitamon Ultra (DAP,

thiamine and yeast cell walls) at the levels normally recommended for grape wine production (0.4-0.5 g L^{-1}) were the best nutrient condition to improve the fermentation characteristics and the formation of desirable non-volatile and volatile compounds in the YPF wines for most yeast strains mentioned above.

The results also showed that the EC1118 with the addition of 0.25 g L^{-1} DAP and 0.5 g L^{-1} Vitamon Combi appeared to be a high producer of varietal volatile thiols, 3-sulphanylhexanol (3SH) and acetic acid 3-sulphanylhexyl ester (3SHA). Nevertheless, it has to be taken into account that DAP addition resulted in excessive formation of keto acids by strain X5. Strain LittoLevure also formed the highest amounts of 3SHA in the 0.5 g L^{-1} Vitamon Combi treatment. Yeast strain X5 with the addition of Vitamon Combi and Vitamon Ultra at 0.4 g L^{-1} also produced the YPF wine having the greatest concentration of 3SH. Neither the yeast strains (EC1118 and X5) nor the nutrient sources (Vitamon Combi and Vitamon Ultra) influenced the level of 3SHA in YPF wines. Concentrations of 3SH and 3SHA were quite beyond their aroma threshold contributing to typical and varietal aromas.

It can be concluded that certain yeast strains and optimal nutrient supplementations had a great impact on successful YPF wine fermentation, minimizing the formation of SO_2-binding and undesirable volatile sulphur compounds and improving desirable aroma compounds in final YPF wine products. Nevertheless, the choice of nutrient supplements and their concentrations is yeast strain-dependent.

This study has an important implication for the YPF wine as well as for the tropical fruit winemaking industry, particularly in Thailand, where a better understanding of the nutritional requirements of *Saccharomyces* yeast is necessary to reduce fermentation problems and to improve the final product quality. It is worthwhile pointing out that this work is an interesting new observation for the YPF wine fermentation, although the fermentation trials were only done in the laboratory scale. Thus, some different fermentation parameters as well as volatile compounds might be achieved under industrial conditions. Nevertheless, a better understanding of the effect of nutrient supplementation on yeast metabolic products as well as sensory properties is still required in further work in both the laboratory and pilot scale.

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Abbreviations and symbols

BzEtAc	Benzene acetic acid ethyl ester
BuEtE	Butanoic acid ethyl ester
iBuEtE	Isobutanoic acid ethyl ester
CS_2	Carbon disulphide
d	Day
DAEtE	Decanoic acid ethyl ester
DAP	Diammonium hydrogen phosphate
DEDS	Diethyl disulphide
DMDC	Dimethyl dicarbonate
DMDS	Dimethyl disulphide
DMS	Dimethyl sulphide
DMTS	Dimethyl trisulphide
^oC	Degree celsius
CO_2	Carbon dioxide
EC	Enzyme Commission
EMS	Ethyl methyl sulphide
EtAc	Acetic acid ethyl ester
EtSAc	Thioacetic acid S- ethyl ester
EtSH	Ethanethiol
FAN	Free alpha amino nitrogen
FTIR	Fourier transform infrared spectroscopy
g	Gram
μg	Microgram
GC-MS	Gas chromatography-mass spectrometry
HexAc	Acetic acid hexyl ester
HAEtE	Hexanoic acid ethyl ester
H_2S	Hydrogen Sulphide
i.d.	Internal diameter
IDY	Inactive dry yeast
Kg	Kilogram
KMS	Potassium metabisulphite
L	Litre
L^{-1}	per Litre
μL	Microlitre
LAEtE	Lactic acid ethyl ester

Abbreviations and symbols (continued)

2MeBuAc	Acetic acid 2-methylbutyl ester
3MeBuAc	Acetic acid 3-methylbutyl ester
m	Metre
mg	Milligram
min	Minute
min^{-1}	per minute
ml	Millilitre
mm	Millimetre
μm	Micrometre
MCFA(s)	Medium-chain fatty acid(s)
MeSAc	Thioacetic acid S-methyl ester
MeSH	Methanethiol
4,2,2MSB	4-Methoxy-2-methyl-2-sulphanylbutane
4MSP	4-Methyl-4-sulphanylpentan-2-one
N	Nitrogen
NCR	Nitrogen catabolite repression
n.d.	not detectable
ng	Nanogram
n.q.	not quantifiable/ trace
NOPA	*o*-Phthaldialdehyde/N-acetyl-L-cysteine spectrometric assay
OAEtE	Octanoic acid ethyl ester
2PheEtAc	Acetic acid 2-phenyl ethyl ester
PrEtE	Propionic acid ethyl ester
sec^{-1}	per second
3SH	3-Sulphanylhexanol
3SHA	Acetic acid 3-sulphanylhexyl ester
SO_2	Sulphur dioxide
SAdiEtE	Succinic acid diethyl ester
TA	Total acidity
TSS	Total soluble solid
v/v	volume by volume
YPF	Yellow passion fruit

1. INTRODUCTION

1.1 Statement and significance of the study

Grape wine flavour is formed from up to several hundreds of volatile compounds at concentrations ranging from mg L^{-1} to a few ng L^{-1} or even less. The olfactory impact depends on the concentration type and perception value. There are diverse mechanisms involved in the development of the aroma compounds. Varietal aromas have a very high impact and are formed by grape metabolism, depending on the variety, as well as soil, climate and vineyard management techniques. These compounds occur mainly as non-volatile precursors in grapes. Glycosylated forms are much more common than free forms, especially among the monoterpenes. The bound aroma compounds were released by the activity of β-glucosidases that occur in grapes and yeasts during the wine making process (Rapp, 1998; Ribéreau-Gayon et al., 2006 b).

Other key varietal compounds are volatile thiols (thiol referring to the SH functional group) that derive from non-volatile precursors during the alcoholic process due to the bioconservation of yeasts. The volatile thiols, 4-methyl-4-sulphanylpentan-2-one (4MSP) (so-called 4-mercapto-4-methylpentan-2-one, 4MMP), 4-methyl-4-sulphanylpentan-2-ol (4MSPOH) (so-called 4-mercapto-4-methylpentan-2-ol, 4MMPOH), 3-sulphanylhexanol (3SH) (so-called 3-mercaptohexanol, 3MH) and acetic acid 3-sulphanylhexyl ester (3SHA) (so-called acetic acid 3-mercaptohexyl ester, 3MHA) are of particular importance to the wine aroma. 4MSP, 4MSPOH and 3SH are released from S-cysteine conjugates (Bouchilloux et al, 1998; Tominaga et al., 1998 a, 1998 b; Dubourdieu et al., 2006). Swiegers et al. (2005 b) reported that 3SHA is formed by yeast from 3SH by the action of the ester forming alcohol acetyltransferase during alcoholic fermentation. Volatile thiols have extremely low odour thresholds in model solution, e.g. 0.8 ng L^{-1} 4MSP, 55 ng L^{-1} 4MSPOH, 60 ng L^{-1} 3SH and 4 ng L^{-1} 3SHA (Swiegers et al., 2005 a, 2005 b; Dubourdieu et al., 2006). These thiols occur in nearly all varieties, but they are so-called character impact compounds in Sauvignon blanc, Viognier, Scheurebe, Kerner, etc. The 4MSP thiol contributes to flavours in wine that were described as blackcurrant, box tree and broom aromas (e.g. in Sauvignon blanc, Muscat d' Alsace and also sometimes in Riesling). 3SH is mainly responsible for grapefruit and tropical fruit nuances and contributes intensively to the bouquet of Gewürztraminer, Muscat d'Alsace, Pinot gris, Riesling, Manseng and botrytized Sémillon (Dubourdieu et al., 2006; Ribéreau-Gayon et al., 2006 b). 3SHA contributes to boxwood, grapefruit zest and passion fruit aromas (Bouchilloux et al, 1998; Dubourdieu et al., 2006; Ribéreau-Gayon et al., 2006 b; King et al., 2008).

Tominaga et al. (1998 a, 1998 b) and Tominaga & Dubourdieu (2000) demonstrated that cysteine-S-conjugate β-lyases are responsible for the release of the volatile thiols during alcoholic fermentation. Research work of Murat et al. (2001 a) and Dubourdieu et al. (2006) indicated that yeast strains vary in the ability to release 4MSP, 3SH and 4MSPOH. It could be also demonstrated that commercial yeast strains have different enzyme activities for the release of thiols. Therefore, specific yeast strains are selected to enhance the varietal flavours during wine production.

S-(Sulphur-)containing compounds, especially volatile thiols, belong to the most important aroma compounds in food. They are biosynthesised in various plants, especially in tropical fruits, but they are also generated during the fermentation process of alcoholic beverages and in the course of the thermal treatment of food. Yellow passion fruit is a typical example of a fruit, of which the flavour is established by S-containing compounds (Winter et al., 1976; Weber et al., 1994, 1995; Engel, 1999; Blank, 2002; Wakabayashi, 2004). 3SH was firstly identified in passion fruit by Engel & Tressl (1991). Thiols and other S-compounds also contribute to the flavour of pineapple, lychee fruit, blackcurrant, mango, citrus peel, etc. (Brat et al., 2004; Tokitomo et al., 2005). The interest in the biogenesis of these compounds, their release from non-volatile precursors by enzymes and their preservation during food processing and alcoholic fermentation makes the research on thiols and other S-compounds to optimise food quality to one of the most interesting flavour research areas.

In Thailand, purple, yellow and hybrid variety passion fruits are grown commercially. They easily grow at all climate conditions of Thailand. There is a high yield and passion fruits are resistant to diseases. The yellow passion fruit is the most cultivated and has some advantages in comparison to the purple passion fruit like larger fruits, greater yield, more attractive colouring, unique flavour properties and much higher acidity (Vera et al., 2003; www.gpo.or.th/rdi/html/passionfruit.html, 2009; www.moac.go.th/builder/bhad/passionfruit.php, 2009; www.stou.ac.th/study/sumrit/12-51(500)/page1-12-51(500).html, 2009). Frozen juice can be kept without deterioration for one year at 0 ºF (-17.78 ºC) remaining a very appealing product (www.hort.purdue.edu/newcrop/morton/passionfruit.html#Storage, 2009). Passion fruit is a good source of pro-vitamin A, ascorbic acid, riboflavin and niacin and has also a high mineral content. Its distinctive aroma and flavour make it a popular additive to many tropical fruit beverages and food blends, especially pineapple wine and juice. Now, passion fruit is considered to be the high potential fruit for the food and beverage industry with a growing demand, not only as an exotic aroma enhancer but also because of its natural and healthy nutritional value.

It is well-known that S-compounds can also be responsible for certain off-flavours in wine. Various research groups tried to detect undesired S-compounds for off-flavours in grape wines that mainly occur through yeast metabolism during the fermentation process or in the

bottled wines during storage (Rauhut, 1996, 2003; Rauhut et al., 2005 a; Ribéreau-Gayon et al., 2006 a, 2006 b; Fedrizzi et al., 2007). Intensive research work demonstrated that one of the main causes for off-flavours occurring after fermentation is the chosen yeast strain (*Saccharomyces cerevisiae*) and its nutrient requirements and also the nutrient content in the grape musts (Henschke & Jiranek, 1991; Rauhut & Kürbel, 1994; Jiranek et al., 1995 a, 1995 b; Rauhut, 1996; Rauhut et al., 1995, 2000 a, 2000 b). Under nutritionally sufficient conditions, hydrogen sulphide (H_2S) production is normally matched to biosynthetic demand through tight metabolic regulation. A nitrogen deficiency leads to an overproduction of H_2S, which is reminiscent of rotten eggs, and also other disagreeable volatile S-compounds. The intensity and the sensory impression of the resulting off-flavours depend on the qualitative and quantitative compositions of the volatile S-substances in the wines. The formation of S-compounds is also influenced by different requirements of commercial yeast strains for certain amino acids and their capability to produce aroma-active S-substances. It could be demonstrated that yeast strains differ in their formation of volatile S-compounds (Rauhut & Kürbel, 1994; Rauhut et al., 1995, 1997, 2000 a, 2000 b; Rauhut, 2003; Wang et al., 2003; Howell et al., 2005; Edwards & Bohlscheid, 2007; Rauhut, 2009).

Different research groups observed a decrease of nutrients in grape musts due to global climate change and lack of water at specific phases during the vegetation period. This is the main cause for stuck fermentations and off-flavours due to an accelerated formation of S-compounds (methanthiol, ethanthiol, dimethyl sulphide, diethyl disulphide and dimethyl trisulphide, thioacetic acid methyl ester and thioacetic acid ethyl ester etc.) during the last twenty years (Rauhut & Kürbel, 1994; Rauhut et al., 1995, 2005 b; Rauhut, 2009).

The wine makers are allowed to compensate nutrient deficiencies by the addition of ammonium salts, thiamine, yeast cell hulls and inactive dry yeast. Inactive yeasts are more and more used to add micronutrients in a yeast-available form. The additions help to avoid and to reduce off-flavours, but the additions are not often well-balanced and managed (Pozo-Bayón et al., 2009 a, 2009 b). Therefore, a lot of research is being conducted to optimize the supplementation of nutrients. The addition of nutrients is also effecting the formation of higher alcohols and esters that are produced by yeasts during fermentation. These compounds are responsible for the fermentation bouquet and the overall flavour of wines. For that reason an optimised management for the choice of the yeast strains and the addition of nutrient supplements has to be developed to improve also the varietal aroma that can be covered and masked by off-flavour S-compounds or by extreme production of unpleasant higher alcohols or large amounts of esters like acetic acid ethyl ester (Wang et al., 2003; Hernández-Orte et al., 2005, 2006 a; Swiegers et al., 2005 a, 2005 b). A reoccurrence of off-flavours in wines during storage after treatment and bottling is related to a release of unpleasant volatile compounds from non-volatile or volatile precursors like the hydrolysis of

thioacetic acid esters to thiols and acetic acid. Rauhut (1996) also reported that an accelerated formation of H_2S leads to an increase of thioacetic acid esters, which have aroma threshold values > 40 mg L^{-1} (Rauhut, 1993, 1996, 2003; Fedrizzi et al., 2007; Rauhut, 2009).

It is a long tradition in grape wine production getting rid of an off-flavour that is caused by volatile sulphur-compounds by using aeration or by adding of copper sulphate. In the presence of oxygen, H_2S and thiols can be oxidised. Thiols (mercaptans) are oxidised to disulphides which are also odour active S-compounds. Therefore, the aeration can only be used for weak off-flavours. For stronger off-flavours a treatment with copper ions (addition of copper sulphate) is a common practice. Unfortunately, copper ions only react with H_2S and thiols, other S-compounds will remain. Those wines are reduced in their quality causing an economic loss for wine producers. An unsolved problem for wine makers is the removal of undesirable S-substances in wines, because an aeration or a treatment with copper ions will also eliminate or reduce the desirable thiols, which will also be oxidised or react with the added copper ions (Rauhut, 1993, 1996, 2003, 2009). Furthermore, there is a tendency to avoid additions of fining compounds as much as possible and to force minimal processing in wine making (Rauhut, 2003, 2009).

Finally, it could be pointed out that volatile thiols play a major role in the varietal aroma of certain grape varieties as well as in tropical fruits, their juices and wines. It could be also indicated that there is an intensive research going on to release thiols in grapes from certain varieties, but there is nearly no information about optimising the release of thiols in tropical fruit wines. The production of tropical fruit wines will be more and more important for fruit wine industry in Thailand, especially the use of passion fruit should be optimised and increased. Furthermore, there is a lack of knowledge about how the thiols can be conserved after fermentation and how off-flavours caused by undesirable S-compounds can be avoided or removed without diminishing the varietal thiols. The optimisation has to be in line with the treatments that are allowed by the government for the production of fruit wines in Thailand. For example, it is not allowed to use copper sulphate as a fining agent to remove off-flavours caused by S-compounds in fruit wines. Therefore, it is extremely important to improve the release of thiols and the fermentation conditions to avoid off-flavours in tropical fruit wines, especially if further treatments with fining agents are not allowed.

1.2 Objectives

The overall objective of this study is focused on the improvement of varietal and important aromas in grape wines as well as in yellow passion fruit wine by an optimal choice of yeasts and nutrient supplements.
In particular the improvement of aromas in grape wine and yellow passion fruit wine the following objectives are aimed:

- Improving desirable aroma compounds in grape wines from two varieties of Sauvignon blanc and Scheurebe by an optimal choice of *Saccharomyces* yeast strains and nutrient supplements to avoid off-flavours by undesired thiols due to yeast metabolism under nutrient deficiencies

- The fermentation of passion fruit wine at laboratory scale on the basis of the experimental results which were obtained from the research on grape wines

- Recommendations for an adequate choice of commercial yeast strains and/or nutrient supplements to improve fermentation characteristics and varietal aromas in yellow passion fruit wine

2. LITERATURE REVIEW

2.1 Yeasts

Yeasts are defined as unicellular microorganisms classified in the kingdom of fungi, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae as seen in most molds (Kurtzman & Fell, 2006; Fugelsang & Edwards, 2007). According to the phylogenetic diversity, yeasts are shown by their placement in both divisions Ascomycota and Basidiomycota, they mostly reproduce asexually by budding, although in a few cases by binary fission (Barnett et al., 2000; http://lfbisson.ucdavis.edu/PDF/VEN124%20Section%203.pdf, 2001; Boekhout & Phaff, 2003; Jay et al., 2005; Kurtzman & Fell, 2006; Ribéreau-Gayon et al., 2006 a). They are capable of forming sexual states that are not enclosed in a fruiting body (Boekhout & Kurtzman, 1996). From the 100 yeast genera representing approximately 678 (Barnett et al., 2000) and 1,500 species, (Kurtzman & Fell, 2006) that have been described, 15 species are associated with winemaking (Kurtzman, 1998). Regarding the morphology, yeasts can be differentiated from bacteria by their larger cell size and their oval, elongated, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 µm in diameter, although some yeasts can reach over 40 µm. Older yeast cell cultures tend to have smaller cells (Barnett et al., 2000; Walker et al., 2002; Jay et al., 2005). Yeasts produce many colours of colonies, ranging from creamy, to pink, to red (Jay et al., 2005).

Yeasts can grow over a temperature range of 10 to 37 °C, with an optimal temperature range from 30 to 37 °C, depending on the type of species (*S. cerevisiae* works best at about 30 °C). At temperatures above 37 °C yeast cells become stressed and will not divide properly (Jay et al., 2005). They can also grow over wide ranges of acid pH and in up to 21 % ethanol and also in the presence of 55-60 % sucrose (Thomas & Ingledew, 1992; Barnett et al., 2000). Yeasts are chemoorganotrophs as they use organic compounds as a source of energy and do not require sunlight to grow. The main source of carbon is obtained by hexose sugars such as glucose and fructose, or disaccharides such as sucrose and maltose (Fugelsang & Edwards, 2007). Some species can metabolize pentose sugars like ribose (Barnett, 1975), alcohols, and organic acids maltose (Dequin et al., 2003; Jay et al., 2005; Fugelsang & Edwards, 2007). Yeasts are very versatile and some are suitable for industrial purposes. Furthermore, yeasts are easier and cheaper to harvest than bacteria and large-production is not usually liable to evoke the same concern for problems of public health (Barnett et al., 2000).

Yeasts are the most important microorganisms ever exploited by man, because they have been used during several thousands of years for the production of a wide range of food (Fröhlich-Wyder, 2003). In addition to baking and traditional alcoholic fermentations, yeasts are already being used for diverse industrial purposes. Yeasts have been used in several applications: (i) the fermentation of lactose to ethanol, to produce lactose-free milk for sufferers from lactose intolerance; (ii) the production of various alditols, such as glycerol or D-glucitol; (iii) the production of protein from alkanes and paper-pulp waste; (iv) the providing of enzymes, such as β-fructofuranosidase (invertase), α- and β-galactosidase and lipase (Barnett et al., 2000); (v) the production of compounds for research purposes, such as, novel carbon-carbon bonds (Itoh et al., 1989) and methyldiols from aldehydes (Fuganti & Grasselli, 1985) and (vi) as biocontrol agents because of their antifungal activity (Barnett et al., 2000; Fleet, 2007). Cell biomass (food and fodder yeasts) (Boze et al., 1992; Buzzini & Vaughan-Martini, 2006), production of ingredients, additives and processing aids for food processing, such as antioxidants, aromas, colours, flavours and vitamins (Abbas, 2006), yeast probiotics (Fleet, 2006), yeast biocatalysts (Strehaiano et al., 2006), are other applications of yeasts. On the other hand, the presence and metabolism of yeasts can also have some detrimental aspects, such as food and beverage spoilage, food allergens, food safety and yeast-related health (Fleet, 1992, 1993; Caruso et al., 2002; Fleet, 2006).

Nomenclature of enological yeasts

The classification and taxonomies of approximately 678 yeasts and 1,500 species have currently been recognized and described by Barnett et al. (2000) and Kurtzman & Fell (2006). Those versatile yeasts, 15 genus of *Saccharomyces* and 19 genera of non-*Saccharomyces*, are associated with winemaking (Fleet, 1998; Kurtzman, 1998; Dittrich & Großmann, 2005; Jolly et al., 2006). Yeasts that are present in uninoculated grape juice are named by different terms such as natural, native, wild, wine or indigenous yeasts (Soden et al., 1999). *S. cerevisiae* is prevalent on the surface of winery equipment, whereas the indigenous wine yeasts on grapes are considered to be non-*Saccharomyces* species (Fleet & Heard, 1993; von Wallbrunn, 2007). Therefore, in wine production, yeast species may be divided into two broad groups, i.e. *Saccharomyces* and non-*Saccharomyces* groups (Jolly et al., 2006; von Wallbrunn, 2007).

Saccharomyces yeasts are unicellular, globose, and ellipsoid to elongated in shape. Multilateral (multipolar) budding is typical for vegetative reproduction (Vaughan-Martini & Martini, 1998; Ribéreau-Gayon et al., 2006 a). A number of 16 species is characterized under the genus *Saccharomyces* as excellently proposed by Barnett et al. (2000). Nomenclature of *Saccharomyces* yeasts according to Barnett et al. (2000) with the teleomorphic (perfect) names and two anamorphic (imperfect) names is given in **Table 2-1**.

Table 2-1 List of the *Saccharomyces* yeasts, with their teleomorphic and anamorphic names

Teleomorphic name (sexual state)	Anamorphic name (asexual state)
Saccharomyces barnettii	
Saccharomyces bayanus	
Saccharomyces castellii	
Saccharomyces cerevisiae	*Candida robusta*
Saccharomyces dairenensis	
Saccharomyces exiguus	*Candida holmii*
Saccharomyces kluyveri	
Saccharomyces kunashirensis	
Saccharomyces martiniae	
Saccharomyces paradoxus	
Saccharomyces pastorianus	
Saccharomyces rosinii	
Saccharomyces servazzii	
Saccharomyces spencerorum	
Saccharomyces transvaalensis	
Saccharomyces unisporus	

The origin and importance of enological yeasts

Originally, all wine was made by taking advantage of natural microflora for spontaneous fermentation and no deliberate inoculation was made to start the process (Pretorius, 2000). At present, it is recognized that wine fermentation involves the development and activity of a number of different yeast species that originate from grape. Also the indigenous microbiota associated with winery environment participates in these natural wine fermentations (Fleet & Heard, 1993; Heard, 1999). Although, grapes have long been considered the primary source of indigenous wine yeasts (Fleet & Heard, 1993) and are relatively complete in nutrient content, but having a low pH and high sugar content, they exert a strong selective pressure on the microbial species such that only a few yeast species and other microorganisms can proliferate (Henschke, 1997; Dequin et al., 2003).

In alcoholic processes, yeasts are responsible for the biotransformation of fermentable sugars like glucose, fructose and sucrose into alcohol and carbon dioxide via the process of fermentation. They have an enormous impact on wine production (Fleet, 1993; Barnett et al., 2000; Pretorius, 2000; http://lfbisson.ucdavis.edu/PDF/VEN124%20Section%203.pdf, 2001) because: (i) they conduct the alcoholic fermentation; (ii) they can spoil wines during storage in the cellar and after packaging and (iii) they affect wine quality through autolysis. During

wine fermentation not only hexoses are converted by yeasts to ethanol and carbon dioxide, but many compounds are removed from the medium and a large set of by-products are formed that influence the sensory properties of wines (Dequin et al., 2003). The fermentation of wine is mainly conducted by yeast of the genus *Saccharomyces*, of which the two common species mostly involved are *S. cerevisiae* and *S. bayanus* (Pretorius, 2000; http://lfbisson.ucdavis.edu/PDF/VEN124%20Section%203.pdf, 2001; Dequin et al., 2003; Ribéreau-Gayon et al., 2006 a; Fugelsang & Edwards, 2007). In addition to *S. cerevisiae* and *S. bayanus*, it is now well established that various species of non-*Saccharomyces*, such as *Hanseniaspora (Kloeckera), Candida, Pichia, Metschnikowia, Kluyveromyces, Schizosaccharomyces* and *Issatchenkia* can make positive contributions to the fermentation of wine from grapes (Fleet, 1998; Pretorius, 2000; Fleet, 2003; Clemente-Jimenez et al., 2005; Mendoza et al., 2007; Chomsri, 2008; Moreira et al., 2008).

In traditional winemaking, spontaneous fermentation of grape must is performed by a sequential development of different yeast species that originate from the grape and the winery equipment, such as *Hanseniaspora* (*Kloeckera*) *spp*., *Candida* (e.g. *C. stellata* and *C. pulcherrima*), *Brettanomyces* (*B. anomalus* and *B. bruxellensis*), *Cryptococcus*, *Kluyveromyces*, *Metschnikowia* (*M. pulcherrima*, the perfect form of *C. pulcherrima*), *Pichia* (*P. membranifaciens*) as well as species previously assigned to the *Hansenula* genus, e.g. *H. anomala*, the pink yeast *Rhodotorula* (*Rh. minuta*) (Fleet & Heard, 1993; Pretorius, 2000; Romano et al., 2006). However, their growth is generally limited to the first three or four days of fermentation, after which they die. Subsequently, the most strongly fermenting and more ethanol tolerant species of *Saccharomyces* take over and finish the fermentation process (Fleet & Heard, 1993; Martini, 1993). Recently, there has been a re-evaluation of the role of non-*Saccharomyces* yeasts in winemaking (Fleet & Heard, 1993; Ciani & Maccarelli, 1998; Heard, 1999). In fact, spontaneous fermentations usually take longer than most winemakers are willing to accept and the outcome is always not what was anticipated, because it depends not only on the number and diversity of yeasts present in must, but also upon grape chemistry and processing protocol (Fugelsang & Edwards, 2007). At one extreme are those who continue to use solely indigeneous yeasts, believing that unique contributions of diverse yeast species confer a complexity to the wine not seen in inoculated and controlled fermentations. Others prefer to begin with native yeasts and later inoculate with a commercial yeast starter because the wines produced by inoculation were of consistent acceptable quality (Fleet & Heard, 1993; Pretorius, 2000). In the last 30 years, most of the wine industry has tended to move away from spontaneous fermentations towards controlled fermentations initiated by inoculation that are more reliable and facilitate wine production (Dequin et al., 2003; Sablayrolles, 2009).

Impact of enological yeasts on wine fermentation

When must is used as a culture medium, selective pressures always favour the yeasts with the most efficient fermentative catabolism, particulary strains of *S. cerevisiae* and perhaps strains of closely related species such as *S. bayanus* (Henschke, 1997; Pretorius, 2000). For this reason, *S. cerevisiae* is the almost universally preferred yeast for initiating alcoholic fermentation and is often designed as the "wine yeast" (Fleet & Heard, 1993; Martini, 1993; Dequin et al., 2003; Pretorius, 2000). The primary role of wine yeast is to catalyse not only the rapid complete and efficient conversion of grape sugars to ethanol, carbon dioxide and other minor, but also sensorially important metabolites without the development of off-flavours (**Figure 2-1**) (Pretorius, 2000; Swiegers et al., 2005 a). A secondary role concerns the modification and/or release of grape-derived components, such as glyco- and cysteine-conjugate precursors, which enhance the wines' varietal character (Howell et al., 2004, 2005; Swiegers et al., 2005 a, 2005 b).

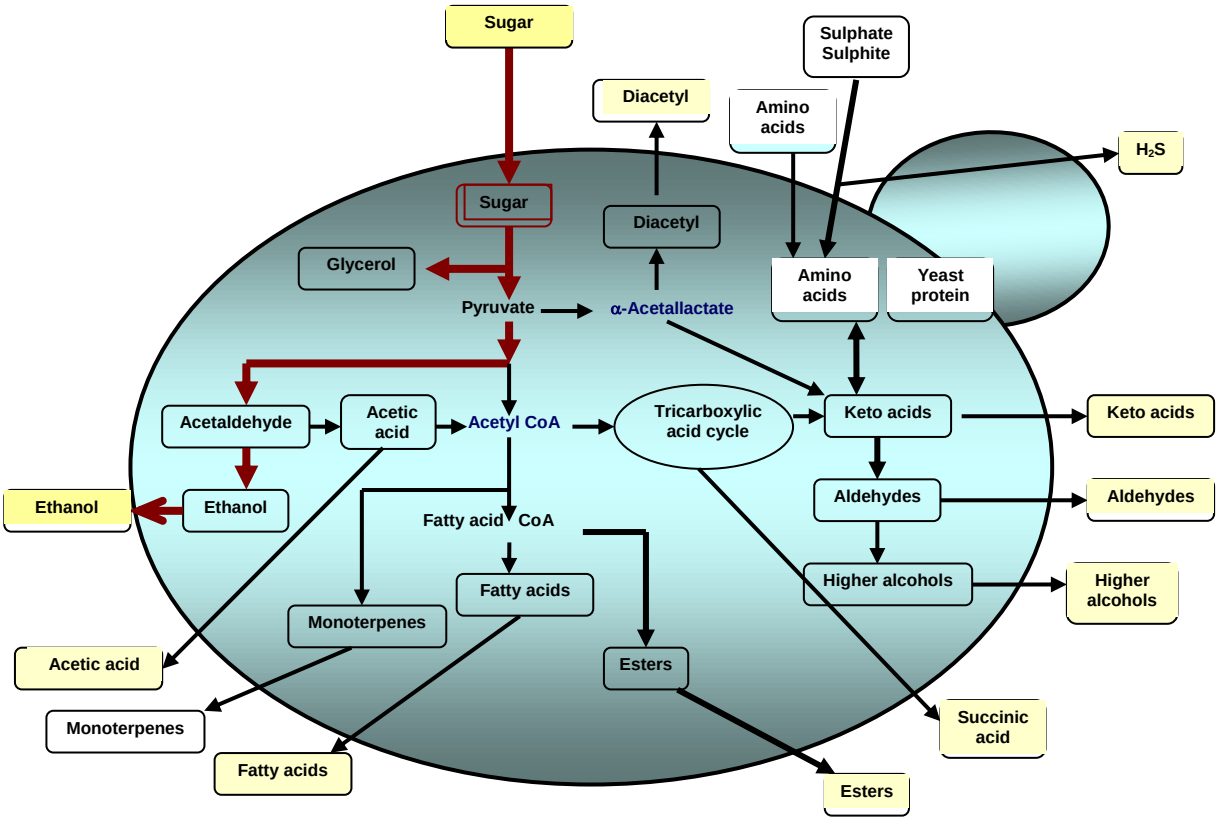


Figure 2-1 A schematic representation of derivation and synthesis of flavour-active compounds from sugar, amino acids and sulphur metabolism by wine yeast

Source: Adapted from Pretorius (2000) and Swiegers et al. (2005 a) and modified

Overall, the goal of using a starter culture is to initiate fermentation as quickly as possible while limiting the potential for spoilage by establishment of numerical dominancy over native species (Fugelsang & Edwards, 2007). The assignment of most of the traditional wine yeast strains to a single species does not, however, imply that all strains of *S. cerevisiae* are equally suitable for the various wine fermentations, they differ significantly in their fermentation performance and their contribution to the final bouquet as well as production of H_2S and quality of wine (Jiranek et al., 1995 b; Pretorius et al., 1999; Swiegers et al., 2005 a). Sablayrolles (2009) reported that more than 200 different *S. cerevisiae* strains are currently commercially available, with highly diverse fermentation properties.

Fermentation predictability and wine quality are directly dependent on wine yeast attributes that assist in the rapid establishment of numerical and metabolic dominance in the early phase of wine fermentation, and that determine the ability to conduct an even and efficient fermentation with a desirable residual sugar level. A wide range of factors affect the fermentation performance of wine yeasts. Apart from a successful inoculation with the appropriate starter culture strain, the physiological condition of such an active dried wine yeast, and its ability to adapt to and cope with nutritional deficiency and the presence of inhibitory substances, are of vital importance to the fermentation performance (Pretorius, 2000). The choice of yeast strain used by the winemaker is increasingly motivated by the potential impact of that strain on the wine characteristics. The very large number of strains commercially available, and many complex mechanisms of interaction between strains, must and fermentation conditions make this choice difficult. The potential of yeast strains to increase the geographical typicity of a wine remains a matter of debate, but specific strains are now widely recognised to be useful: (i) for increasing the fruity character (Torija et al., 2003 a; Sablayrolles, 2009), (ii) for improving some varietal characters in some wines (Delcroix et al., 1994; Murat et al., 2001 a; Swiegers et al., 2005 b, 2006, 2007; King et al., 2008), (iii) for limiting the production of organic acids or increasing the production of glycerol (Scanes et al., 1998), and (iv) for limiting off-flavours, including those due to sulphur (Rauhut, 1993; Rauhut et al., 1996, 1997) and volatile phenols (Shinohara et al., 2000). The use of different *Saccharomyces* strains for wine fermentations resulted in wines with different volatile profiles, through varied relative concentration of acetic acid esters, fatty acid ethyl esters, higher alcohols and wine compositions (Henick-Kling et al., 1998; Antonelli et al. 1999; Heard, 1999; Howell et al., 2006; Chomsri, 2008; King et al., 2008; Swiegers et al., 2009) as well as volatile thiols (Howell et al., 2005; King et al., 2008; Swiegers et al., 2009). Studies have also investigated the effect of simultaneous inoculation and coinoculation with yeast strains to conduct fermentations of wines (Großmann et al., 1996; Howell et al., 2005, 2006; Chomsri, 2008; King et al., 2008; Viana et al., 2009).

2.2 Nitrogen containing compounds in winemaking

Nitrogen containing compounds, e.g. ammonia, amino acids, peptides, polypeptides and proteins, are commonly found in living organisms and are important constituents of food. They supply the required building blocks for protein biosynthesis. In addition, they directly contribute to the flavour of food and are precursors for aroma compounds and colours formed during thermal or enzymatic reactions in production, processing and storage of food (Belitz et al., 2004). Many microorganisms have the ability to use a variety of nitrogen containing compounds as the sole source of all cellular nitrogen (Cooper, 1982).

Sacchromyces cerevisiae can grow in a wide variety of nitrogen-containing substrates (Cooper, 1982; Large, 1986). It preferentially uses simple nitrogen sources such as ammonium ions and free alpha amino acids (Cooper, 1982; Henschke & Jiranek, 1993; Jiranek et al., 1995 a; Valero et al., 2003). However, the secondary amino acids, such as proline and hydroxyproline, are not metabolized to any great extent under usual winemaking conditions (Duteurtre et al., 1971; Ingledew et al., 1987). Low molecular weight peptides can also be used but grape proteins cannot be used as a source of nitrogen since *S. cerevisiae* lacks significantly of extracellular proteolytic activity. Therefore, the usable nitrogen fraction is often referred to as yeast assimilable nitrogen (YAN). The remaining compounds of total nitrogen, which includes proline and hydroxyproline, larger molecular weight peptides and protein, will be reffered to as yeast non-assimilable nitrogen (YNAN) (Bell & Henschke, 2005). The consumption rate and metabolism of nitrogenous compounds depend on the yeast strain, its physiological state and the physicochemical properties of the must or wine. *S. cerevisiae* can either directly incorporate amino acids into the proteins, or use them as a source of nitrogen by oxidative deamination (except lysine and histidine); alternatively, an amino acid is degraded by liberating nitrogen for the biosynthesis of other nitrogenous cell constituents, and its carbon structure can be excreted to the wine or be used as a carbon source for the biosynthesis of other compounds (Large, 1986).

Wine is a complex mixture of organic molecules that are present in an extremely wide range of concentrations. Of those molecules, nitrogen containing compounds are of great interest (Valero et al., 2003). Quantitatively, next to sugars, nitrogenous compounds are the most important nutrient substances found in grape must (particularly ammonium ion, amino acids, peptides, and small polypeptides) that can be used as nitrogen sources by yeasts (Mauricio et al., 1995; Dharmadhikari, 2001; Torija et al., 2003 b). On a dry weight basis, about 10 % of yeast weight consists of nitrogen. All the nitrogen used in building cellular material (population 10^8 cells ml^{-1}) during fermentation is taken from the must. It is therefore important that the must contains sufficient amounts of nitrogen to support a healthy yeast population during fermentation (Dharmadhikari, 2001). Nitrogen containing compounds in must and

juice play important roles in fermentations of wine as it is a necessary nutrient for yeast growth and metabolism. Nitrogen deficiencies in grape must and juices is one of the principal factors limiting growth and sugar attenuation (Hernández-Orte et al., 2006 a), and may result in sluggish or stuck fermentations (Salmon, 1989; Kunkee, 1991; Blateyron & Sablayrolles, 2001; Mendes-Ferreira et al., 2007 a, 2007 b). These compounds are known to be essential to the vinification process, not only because they influence growth and metabolic activity of the yeasts but also because they affect the formation of higher alcohols, which contribute to the aroma of wine and hence to its quality (Mauricio et al., 1995; Valero et al., 2003). Like proteins, they also influence clarification, microbial instability (Bell & Henschke, 2005) and wine stability, particularly in white wines (Dharmadhikari, 2001). Low levels of yeast assimilable nitrogenous compounds have been related to lower fermentation rates, longer fermentations (Bely et al., 1990) and cellular activity. Others consider this condition as a cause for low resultant biomass concentrations (Bisson, 1991; Cramer et al., 2002; Ribéreau-Gayon et al., 2006 a). These compounds are also important fermentation activators, since upon their depletion the rate of fermentation can substantially diminish and even cease (Lagunas et al., 1982; Salmon, 1989; Manginot et al., 1998). Limiting YAN is thought to affect yeast by reducing yeast cell multiplication and by decreasing indirectly the rate of glycolysis (Bely et al., 1990). These effects also depend on the nitrogen source, since growth on good nitrogen sources such as ammonia, glutamine and asparagine seems to yield relatively higher growth rates than on poor ones such as proline and urea (ter Schure et al., 2000).

Furthermore, numerous studies about the roles of yeast in the development of wine aroma, flavour and mouth-feel are becoming more clearly defined, as well as the impact of nitrogen on the flavour metabolism of yeasts (Henschke & Jiranek, 1993; Rapp & Versini, 1996; Albers et al., 1996; Bell & Henschke, 2005; Hernández-Orte et al., 2005; Swiegers et al., 2005 a). The limitation of nitrogen can also influence the formation of reduced sulphur compounds, such as hydrogen sulphide (Henschke & Jiranek, 1991; Giudici & Kunkee, 1994; Jiranek et al., 1995 b; Ugliano et al., 2009) and volatile sulphur compounds (Moreira et al., 2002). On the other hand, the degradation of some nitrogen compounds contributes to the formation of a carcinogenic compound, biogenic amines and ethyl carbamate, which are considered to be detrimental to health (Monteiro et al., 1989; Ough, 1991; Zoecklein et al., 1999; Bell & Henschke, 2005). The influence of the nitrogen source (Bisson, 1991; Monteiro & Bisson, 1991; Ough et al., 1991; Monteiro & Bisson, 1992 a, 1992 b; Albers et al., 1996; Torija et al., 2003 b; Wang et al., 2003; Hernández-Orte et al., 2006 a, 2006 b), the amount and timing of nitrogen addition on wine fermentation and volatile compounds has recently been the subject of several studies (Beltran et al., 2005; Rosi et al., 2008). The biological aging process has also received some attention in this respect (Mauricio & Ortega, 1997; Valero et al., 1999). More recently, a research conducted by Osborne & Edwards (2006)

indicated that many yeast strains inhibited the bacteria during fermentation under high nitrogen conditions. Some research also demonstrated the impact of different nitrogen sources on the varietal aroma release, production of 3SH increases when urea is substituted for diammonium phosphate (DAP) as the sole nitrogen source on the synthetic media and grape must complementation with the DAP induces a decrease of 3SH as well (Subileau et al., 2008).

Nitrogen containing compounds in grape juice and wine are consist of an ammonia component and a more complex amino-acid based nitrogen component, e.g. amino acids, oligopeptides, polypeptides, proteins, amide nitrogen, bioamines, nucleic acids, amino sugar nitrogen, pyrazines, vitamins and nitrate (Ough, et al., 1991; Henschke & Jiranek, 1993; Mauricio et al., 1995; Zoecklein et al., 1999; Cramer et al., 2002; Dharmadhikari, 2001; Torija et al., 2003 b). In grape must and wine, concentrations and compositions of these compounds are found in a wide range according to grape variety, rootstock, environment, site, seasonal and growing conditions, level of maturity and juice extraction (Huang & Ough, 1989; Sponholz, 1991; Gockowiak & Henschke, 1992; Monterio & Bisson, 1992 a, 1992 b; Spayd & Andersen-Bagge, 1996; Hernández-Orte et al., 1999; Stines et al., 2000; Swiegers et al., 2005 a). **Table 2-2** lists the individual amino acids commonly found in the whole grape and grape juice and **Table 2-3** lists concentrations of various nitrogen compounds found in grape juice and wine.

Some research reported that the total nitrogen content of grape juice ranges 40-fold from 60-2400 mg L^{-1} and can therefore be growth-limiting (Henschke & Jiranek, 1993; Ribéreau-Gayon et al., 2006 b). A minimum requirement for nitrogen of 120-140 mg N L^{-1} has been widely reported (Bely et al., 1990; Bisson, 1991; Henschke & Jiranek, 1993; Bell & Henschke, 2005). Grape juices with yeast assimilable nitrogen (YAN) concentration below 140 mg N L^{-1} have a high probability of becoming problem ferments due to inadequate yeast growth and poor fermentation activity while a concentration above 400 mg N L^{-1} YAN leads to increased biomass, yeast growth and fermentation performance (Henschke & Jiranek, 1993; Zoecklein et al., 1999; Bell & Henschke, 2005). An inadequacy of nitrogen-containing compounds of grape juices and must for wine fermentation has often been reported. Ribéreau-Gayon et al. (2006 b) described that analytical findings on the extent and frequency of nitrogen deficiencies in Bordeaux grape musts from 1996-2006 vintages were 22 % in white must, 49 % in red must, 60 % in rosé must and 89 % in botrytized musts. Hence, the assessment of the nitrogen requirement for wine yeast should be controlled because it can have an impact on yeast growth, fermentation kinetics and the resulting wine. Regarding the variety of these nitrogen containing compounds, only some compounds found in musts and wines are presented in this review.

Ammonium

The ammonium ion is an important component of must YAN and is one of the most preferred yeast nitrogen sources because it is readily assimilated by the yeasts, and serves as the primary form of available nitrogen for yeast metabolism (up to 40 %) in grape juice (Cooper, 1982; Bisson, 1991; Monteiro & Bisson, 1992 a, 1992 b; Henschke & Jiranek, 1993; Bell, 1994; Ayestarán et al., 1995; Beltran et al., 2004; Vilanova et al., 2007; Boulton et al., 2009). It can influence amino acid metabolism by reducing catabolic enzyme levels and transport activity through various mechanisms, such as nitrogen catabolic repression (Cooper & Sumrada, 1983). It is rapidly consumed at the beginning of the fermentation process. Addition of ammonium salt has a significant effect not only on cell population, the fermentation rate, the production of alcohol, volatile acidity and pH (Monteiro & Bisson, 1992 a; Ayestarán et al., 1995; Bely et al., 2003; Mendes-Ferreira et al., 2004; Taillandier et al., 2007), but also on the formation of metabolic compounds, esters, higher alcohols and volatile sulphur compounds (Ayestarán et al., 1995; Bell & Henschke, 2005; Miller et al., 2007; Ugliano et al., 2007; Vilanova et al., 2007; Rosi et al., 2008; Ugliano et al., 2009; Boulton et al., 2009). Ugliano et al. (2007) reported that overuse of DAP can also stimulate overproduction of acetic acid esters, especially acetic acid ethyl ester, resulting in the perception of volatile acidity and suppression of varietal character. The ammonium concentration of grape berries declines during ripening with an increase in amino acid nitrogen, peptide nitrogen and protein (Bell, 1994; Zoecklein et al., 1999; Bell & Henschke, 2005). The concentration of ammonium widely ranges from 5 to 325 mg N L^{-1} in grapes (Bely et al., 1991; Henschke & Jiranek, 1993; Butzke, 1998; Zoecklein et al., 1999; Carnevillier et al., 2000) and from a few mg L^{-1} to 50 mg L^{-1} in wine.

Amino acids

The primary amino acids constitute a major source of YAN for yeast, however, they vary in their efficiency as nitrogen sources (Cooper, 1982; Jiranek et al., 1995 a). Most of the 20 commonly occurring amino acids are found in grapes and musts (**Table 2-2**). Their concentration and composition can vary according to grape variety, cultivation, rootstock, region and seasonal conditions, level of maturity and processing techniques. L-arginine and L-proline are generally predominant amino acids found in grape and must (Huang & Ough, 1989; Sponholz, 1991; Gockowiak & Henschke, 1992; Spayd & Andersen-Bagge, 1996; Hernández-Orte et al., 1999; Stines et al., 2000; Soufleros et al., 2003; Herbert et al., 2005; Boulton et al., 2009). Many studies of amino acids in must and wine have commonly presented the L-amino acids. D-amino acids have only been examined in a few studies as they have been considered as unnatural amino acids (Brückner & Westhauser, 2003; Brückner & Pätzold, 2006; Pätzold & Brückner, 2007).

The amino acids in grape juice and must are generally present in the range of 170-4000 mg L^{-1}, while in wines in the range of 3-4000 mg L^{-1} (Carnevillier et al., 2000; Ribéreau-Gayon et al., 2006 b; Boulton et al., 2009). The individual amino acid commonly found in the whole grape and grape juice varies significantly (**Table 2-2**). Arginine is quantitatively the most important amino acid utilizable by *Saccharomyces* in grapes (Monteiro & Bisson, 1992 a; Henschke & Jiranek, 1993; Bell, 1994; Jiranek et al., 1995 a; Stines et al., 2000; Bell & Henschke; 2005; Boulton et al., 2009), due to its catabolism by arginase to form L-ornithine and urea, which under appropriate conditions ultimately form glutamate and ammonium, respectively, the latter two compounds are precursors for general amino acid biosynthesis in yeast (Large, 1986). Arginine is rapidly incorporated by yeast at the start of fermentation and subsequently released back into the wine during autolytic cycles (Fugelsang & Edwards, 2007). L-proline was also found at high concentrations, at 750-2257 mg L^{-1} of most cultivars (Huang & Ough, 1989; Spayd & Andersen-Bagge, 1996; Boulton et al., 2009). However, it is only utilised to a limited extent by yeast to the normal anaerobic environment of alcoholic fermentation (Duteurtre et al., 1971; Ingledew et al., 1987), therefore it is referred to as yeast non assimilable nitrogen (YNAN) (Bell & Henschke, 2005).

Regarding the metabolism of yeast, a supplement of amino acids in grape juice could influence the synthesis of cellular protein and, in turn, affect the biomass and fermentation rates (Albers et al., 1998; Salmon & Barre, 1998; Boulton et al., 2009), and shorten fermentation time, leading to high alcohol production (Hernández-Orte et al., 2006 a). Jiranek et al. (1995 a) demonstrated that the kinetics of the utilization of individual amino acids varied between yeast strains. However, arginine, serine, glutamate, threonine, aspartate and lysine typically comprised the bulk of the nitrogen consumed. Miller et al. (2007) reported that the effect of the amino acid addition in Chardonnay juice on amino acid utilization by yeast varied depending on the specific amino acid. The more concentration of amino acid addition in juice leads to the greater utilization of leucine, isoleucine, methionine, phenylalnine, tryptophan, tyrosine and valine, but the less utilization of histidine, glutamine and alanine by yeast. In addition to regulating growth, amino acid availability can also affect many aspects of yeast metabolism, including the formation of volatile and non-volatile compounds that are important for the organoleptic qualities of wine (Albers et al., 1996; Bell & Henschke, 2005; Garde-Cerdán & Ancín-Aypilicueta, 2007).

Table 2-2 The identity and concentration of amino acids found in the whole grape and/or juice at harvest

Amino acid	Concentration range (mg L^{-1})
Alanine	10 - 227
Arginine	20 - 2322
Asparagine	1 - 171
Aspartic acid	10 - 138
Citrulline	0.1 - 83
Cysteine	1 - 8.2
Glutamine	9 - 4499
Glutamic acid	27 - 454
Glycine	1 - 20
Histidine	5 - 197
Isoleucine	1 - 117
Leucine	2 - 160
Lysine	0.7 - 45
Methionine	1 - 33
Ornithine	0.1 - 27.2
Phenylalanine	2.8 - 138
Proline	9 - 2257
Serine	13 - 330
Threonine	9 - 284
Tryptophan	0.2 - 11
Tyrosine	2 - 33
Valine	7 - 116

Source: Huang & Ough (1989), Sponholz (1991), Henschke & Jiranek (1993), Spayd & Andersen-Bagge (1996), Hernández-Orte et al. (1999), Bell & Henschke (2005), Boulton et al. (2009)

The non-volatile compounds, glycerol, malic acid and α-ketoglutarate have been reported to vary according to amino acid, nitrogen source and concentration (Radler, 1993; Albers et al., 1996). The amino acid uptake of yeast influences the aroma generation during alcoholic fermentation (Swiegers et al., 2005 a; Miller et al., 2007). The most aroma compounds include higher alcohols, short to medium-chain fatty acids and their ethyl esters and acetic acid esters (Lambrechts & Pretorius, 2000; Francis & Newton, 2005). Wines obtained from

musts supplemented with amino acids have higher levels of γ-butyrolactone, isobutanol and isobutyric acid (Hernández-Orte et al., 2005). Furthermore, some research showed that the presence of methionine in synthetic medium caused no formation of hydrogen sulphide (Eschenbruch, 1974; Giudici & Kunkee, 1994), but this compound was formed in the presence of cysteine. On the other hand, an experiment carried out by Moreira et al. (2002) suggested that the addition of methionine to grape musts enhanced the production of sulphur compounds and yeast strains also influence the effect of amino addition.

Peptides

Peptides are short polymers formed by binding amino acids together through an amide linkage or peptide linkage and their hydrolysis results in free amino acids. Peptides are denoted by the number of amino acid residues as di-, tri-, tetrapeptides, etc. The term "oligopeptides" is used for those with 10 or less amino acids. Higher molecular weight peptides are called polypeptides and are referred to have molecular weight below 10 kDa (Fukui & Yokotsuka, 2003). Peptides can be substances of great biological importance. They exhibit multiple interesting functional properties, e.g. as antioxidants, antimicrobial agents, surfactants with foaming and emulsifying capabilities (Desportes et al., 2000; Brückner & Koza, 2003; Belitz et al., 2004). It is well established that they contribute to bitter, sweet and umami tastes, such as bitter peptides in cheese or aspartame and a sweet peptide, which is 180 times sweeter than sucrose (Ishibashi et al., 1987, 1988; Polo et al., 1992; Turgeon et al., 1992; Bumberger & Belitz, 1993). They also play a role in the development of some physico-chemical characteristics, flavours and organoleptic properties of wine, like in other foodstuff (Acedo et al., 1994; Desportes et al., 2000).

Peptides are widespread in nature including in grape must and wines. They are major compounds representing together with amino acids the main nitrogen fraction in wines (Desportes et al., 2000). Polypeptides constitute a significant proportion of the total nitrogen content in wine between 20 % and 90 % (Zoecklein et al., 1999). Analyses carried out by Monteiro et al. (2001) revealed that wines contain a large number of distinct polypeptides. Yokotsuka et al. (1975) demonstrated that the peptides in the Koshu Japanese juice mainly consisted of aspartic acid and glutamic acid, which were about 65 % of all the amino acids constituting the peptides. On the other hand, the major amino acids constituting the peptides in the wine were glutamic acid, aspartic acid, glycine and proline. Up to now, most of the studies conducted on wine peptides have been restricted to the determination of the total amino acid composition of wine peptides, the results have been simply obtained by investigating the difference between the amino acid content before and after peptides hydrolysis. Peptides have been rarely studied because their isolation from wine is quite

difficult (Poux & Ournac, 1970; Yokotsuka et al., 1975; Usseglio-Tomasset & Bosia, 1990; Moreno-Arribas et al., 1998 a, 1998 b).

During the first stage of wine fermentation, peptides are assimilated by the yeast together with free amino acids. At the end of fermentation, there is an excretion of free amino acids and small peptides from the yeast to the wine (Ough et al., 1991; Dizy & Polo, 1996). Yokotsuka et al. (1975) showed that compositional changes in peptides occurred during wine making, especially fermentation. Some researches demonstrated that peptides could be released by yeast during base wine preparation and second fermentation of sparkling wine (Martínez-Rodríguez et al., 2002; Moreno-Arribas et al., 1996). The enrichment of wine with short peptides and proteins can be made through addition of yeast autolysate which gives rounder and fuller wines (Feuillat, 2005). Furthermore, Oganesjanz et al. (2007) briefly noted that an increase of nitrogen containing compounds, peptides included, through the addition of yeast lysate in wine improves the organoleptic quality, particularly its complexity and harmony is enhanced. In wine technology, some peptides have shown to be very effective fining agents, at least at the same level as gelatin or soluble poly(vinylpyrrolidone) (PVP) (Yokotsuka & Singleton, 1995).

Among the great number of peptides found in wines, glutathione, a tripeptide γ-L-glutamyl-L-cystinylglycine is perhaps the most intensively studied (Duncan & Derek, 1996; Penninckx, 2002; Roussis et al., 2007). Glutathione is a polypeptide produced by the grapevine and by wine yeast at the end of fermentation. It is a strong antioxidant and helps to stabilize aroma components and plays an important role as an oxidative buffer, thus impacting wine longevity (Zoecklein, 2007). Therefore, it can prevent oxidation of white grape musts (Cheynier et al., 1986; Singleton, 1987; Cheynier et al., 1989). It is also discussed as an anticarcinogenic molecule due to its ready oxidation (Roussis et al., 2007). Cheynier et al. (1989) showed that glutathione ranged from 14-102 mg L^{-1} in grape musts from different varieties. Park et al. (2000 a, 2000 b) found this compound up to 1.3 mg L^{-1} in grape musts and up to 5.1 mg L^{-1} in wines. Du Toit et al. (2007) also detected up to about 35 mg L^{-1} in wines. Final concentration of glutathione in wine was correlated with both total nitrogen and assimilable amino acid concentration and an increase of glutathione towards the end of fermentation was observed. Lavigne et al. (2007) investigated that glutathione in the wine can be increased through the choice of an adequate yeast strain and when the wine is stored on lees. Dubourdieu & Lavigne-Cruège (2002) proposed that glutathione seems to play an important role in protecting volatile thiols that are responsible for the varietal flavours of wines during the ageing of bottled white wines. However, the supplementation of must with glutathione to a concentration of more than 50 mg L^{-1} can lead to unpleasant volatile sulphur compounds under certain conditions (Rauhut et al., 2001; Rauhut, 2003).

In recent years, the organic nitrogen sources, such as commercial preparations that contain yeast derivative products, which also contain lipids and sometimes other nutrients like yeast cell wall, yeast extract, have become commercially available (Munoz & Ingledew, 1990; Belviso et al., 2004). Inactive yeasts, yeast autolysates, yeast extracts and yeast hulls or walls can be included under the generic name of inactive dry yeast (IDY) preparations. Some of the compounds released during yeast autolysis like peptides, amino acids and mannoproteins, seem to be responsible for the great number of applications attributed to these preparations. They can be used as alcoholic fermentation enhancers (Lafon-Lafourcade et al., 1984; Feuillat & Guerreau, 1996; Pozo-Bayón et al., 2009 a, 2009 b), as protective agents to improve active dry yeast rehydration (Caridi et al., 1999; Caridi, 2002) or as organoleptic enhancers stabilizing the colour of red wines by using mannoprotein-rich IDY preparations (Escot et al., 2001; Doco et al., 2003). In fact, yeast derivatives, particularly autolysates, are not completely soluble, and the presence of particulates can be observed when they are added to wines. It is composed of yeast cell wall residues that remain in the growth medium after the lysis treatment, and their ability to bind aroma compounds is well reported in literature (Lubbers et al., 1994 a). Moreover, yeast macromolecules and colloids, released in wine during autolysis, can also determine different sensory effects, interacting with aroma compounds and modulating their volatility and perception (Lubbers et al., 1994 b). Thus, yeast derivatives are used as a source of mannoproteins in winemaking and could affect the aroma intensity, wine oxidation, colour and fruity flavours of treated wines. Comuzzo et al. (2005) found that the lowest addition of yeast derivatives showed an increase in the fruity and flowery perception of some volatile compounds (e.g. esters), while for higher amounts the release of some carboxylic acids characterized by cheese-like and unpleasant odours was observed.

Despite the fact that many of these preparations are currently on the market under different brands, claiming different wine improvements, scientific information about the chemistry behind their use and their action mode is still scarce. Therefore, scientific studies for a better characterization of the changes that these preparations induce in wines are required for the establishment of better criteria for their enological use. For these reasons, the olfactory examination of commercial formulates (to select less odourous and soluble products) and preliminary laboratory tests on small scale could be useful tools to control the effects of the treatment (Comuzzo et al., 2005). Particular problems are associated with attempts to measure peptide in yeasts. Furthermore, wine is a complex matrix and thus many methods for the determination of peptides have to be specifically developed for wine analysis. These methods and extraction techniques are necessary to remove interfering compounds. The applicability, advantages and disadvantages of these methods should be considerably appropriate for grape juice and wine (Chomsri, 2008).

Table 2-3 Concentration of nitrogen containing compounds found in grape juice and wine

Components	Concentration (mg L^{-1})	References
Amides in must and wine	10 - 40, 8 - 35	Carnevillier et al. (2000)
Amino acids		
- grape must and juice	1 - 1130	Boulton et al. (2009), Ferreira et al. (2002)
- grape and wine	1000 - 4000	Ribéreau-Gayon et al. (2006 b)
- wine	130 - 590	Carnevillier et al. (2000)
- Japanese wine	405 - 1559	Yokotsuka et al. (1977)
Assimilable amino acids in musts	331 - 1375	Fukui & Yokotsuka (2003)
Free amino acids in must	32.5	Bell & Henschke (2005)
Free amino nitrogen (FAN)	15 - 230	Zoecklein et al. (1999)
Ammonium		
- grape and must	5 - 325, 10 - 120	Henschke & Jiranek (1993), Carnevillier et al. (2000)
- wine	3 - 30	
Peptides		
- Chardonnay must	7.6 - 20.3 mg g^{-1}	Nakopoulou et al. (2006)
- Chardonnay wine	104.8 - 139	
- Japanese wine	137 - 904	Yokotsuka et al. (1977)
Glutathione		
- grape must	1.3 - 104	Cheynier et al. (1989), Park et al. (2000 a, 2000 b), Du Toit et al. (2007)
- wine	1 - 35	
Nitrogen compounds		
- soluble nitrogen compounds in must	100 - 1000 mg N L^{-1}	Ribéreau-Gayon et al. (2006 b)
- grape	600 - 2400 mg Kg^{-1}	Radler (1993)
- wine	200 -1400	
Total nitrogen		Ribéreau-Gayon et al. (2006 b)
- white wine	70 - 700	
- red wine	77 - 377	
Protein		
- soluble proteins in must	93.5	Bell & Henschke (2005)
- soluble protein in grape juice	118 - 800	Wigan & Decker (2007)
- grape juice	1.5 - 100	Ribéreau-Gayon et al. (2006 b)
- wine	8 - 1000	Bisson (1991), Monteiro et al. (2001), Ribéreau-Gayon et al. (2006 b)
Ethyl carbamate in wine	1 - 7.7 μg L^{-1}	Zoecklein et al. (1999), Ribéreau-Gayon et al. (2006 b)
Urea in wine	< 1	Ribéreau-Gayon et al. (2006 b)
Histamine in wine	0 - 11.3	Zoecklein et al. (1999), Ribéreau-Gayon et al. (2006 b)

Other nitrogen-containing components

Nitrates (NO_3^-) and nitrites (NO_2^-) are present in wines at low levels, usually less than 0.3 % of the total nitrogen. White table wines were found to have 2.1-53.7 mg L^{-1} nitrate, while red wine had 0.9-41.4 mg L^{-1} (Ough & Crowell, 1980). Ough & Amerine (1988) found in German wines nitrate levels less than 7 mg L^{-1}, and 1.65 mg L^{-1} in Italian white wines. Field nitrogen fertilization of the vines has little effect on the residual nitrate in the grape. Fermentation does not alter the nitrate content significantly (Ough & Crowell, 1980). *Saccharomyces* yeast is incapable of utilizing nitrate as a nitrogen source, there are a couple of other yeast species, such *as Candida* and *Hansenula*, that have this capability (http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/03%20Yeast%20Metabolism.pdf, 2009).

Another group of nitrogen compounds present in wines are biogenic amines, which are undesirable compounds produced in food mainly through bacterial decarboxylase activity (Leitão et al., 2000). It is well known that consumption of food containing high amounts of biogenic amines may cause headaches, nausea, cardiac palpitations and digestive problems (Silla-Santos, 1996; Anli et al., 2004). These amines more commonly found in wine are 2-phenylethylamine, putrescine, spermidine, spermine, histamine, tyramine, cadaverine, mercaptoethylamine, ethanolamine and serotonin (Lehtonen, 1996; Leitão et al., 2000; Lonvaud-Funel, 2001; Caruso et al., 2002; Gardini et al., 2005; Ansorge, 2007). They are derived from microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketone by amino acid transaminases (Zolou et al., 2003). Biogenic amines in wines may come from two different sources, i.e. raw materials and fermentation processes (Gardini et al., 2005; Marques et al., 2008). In general, low levels of biogenic amines were found in musts and wines, in comparison to other food, however, biogenic amines can occur in much higher concentrations (Herbert et al., 2005).

Nitrogen-containing flavour compounds are also important in enology. For example, *ortho*-aminoacetophenone (*O*-AAP), the taint compound is responsible for the atypical or untypical ageing (UTA) off-flavour observed in some white wines (Rapp et al., 1993). UTA wines lose their varietal character and begin to exhibit atypical flavours such as “acacia blossom”, “floor polish”, “naphthalene” (mothballs) and” wet towel” (Sponholz et al., 2000; Winter, 2003; Bell & Henschke, 2005). Methyl anthranilate, also known as methyl 2-aminobenzoate or carbomethoxyaniline, is a methyl ester of anthranilic acid or o-aminobenzoic acid, which is related to the “foxy” taste of labrusca grapes and related hybrids (Rapp & Versini, 1996; Rapp, 1998). Additionally, methoxypyrazines, cyclic nitrogen-containing compounds, are reported to be responsible for the vegetative, herbaceous, capsicum-like aromas frequently noted in wines produced from Cabernet Sauvignon and Sauvignon blanc (Sala et al., 2004 a, 2004 b; Bell & Henschke, 2005). The nitrogen containing flavour compounds formed during fermentation may influence the fermentation bouquet (Ough & Amerine, 1988).

Utilization of nitrogen containing compounds by yeasts

A broad range of nitrogenous compounds, e.g. amino acids, ammonium, amines, polyamines, amides, S-adenosylmethionine, nucleotides and nucleic acid derivatives, vitamins, peptides, proteins and trace amounts of nitrates found in grape juice can be metabolized by yeasts (Henschke & Jiranek, 1993; Fugelsang & Edwards, 2007). It is well established that yeasts preferentially utilize certain nitrogen sources in a mixture and that the pattern of nitrogen compounds such as ammonium (inorganic) and amino acids (organic) accumulated depends on the nitrogen composition and concentration of the fermentation substrate (Bell & Henschke, 2005). Within the complexity of nitrogenous components in must and wine, amino acids and ammonium ions are the most important for yeast growth and metabolism (Bisson, 1991; Valero et al., 2003). These compounds are rapidly accumulated by yeast in the early stages of fermentation, during which they fill the biosynthetic pools of amino acids needed for protein synthesis and growth, while the surplus is stored in the cell vacuole (Bisson, 1991; Salmon, 1996). Cooper (1982) also reported that the consumption rate and metabolism of nitrogenous compounds depend on the yeast strain, its physiological state and the physicochemical properties of the must or wine. *S. cerevisiae* can either incorporate amino acids into the proteins directly, or use them as a source of nitrogen by oxidative deamination (except lysine and histidine), alternatively, an amino acid is degraded by liberating nitrogen for the biosynthesis of other nitrogenous cell constituents, and its carbon structure can be excreted to the wine or be used as a carbon source for the biosynthesis of other compounds.

Although, the *S. cerevisiae* wine yeast is able to assimilate various nitrogen containing substrates, the preferred nitrogen sources are ammonium, glutamine and asparagine classified as good nitrogen sources (Cooper, 1982; Large, 1986; Henschke & Jiranek, 1993; Beltran et al., 2004; Bell & Henschke, 2005). In general, nitrogen sources that favour high growth rates are preferentially assimilated because their metabolism readily yields ammonia, glutamate or glutamine, which play a central role in nitrogen metabolism (Dickinson, 2004; Bell & Henschke, 2005). Yeasts use a mechanism called nitrogen catabolite repression (NCR), which mediates the selection of good nitrogen sources by the expression of appropriate transport systems (permeases) and the degradation of non appropriate permeases (ter Schure et al., 2000; Magasanik & Kaiser, 2002; Bell & Henschke, 2005).

Ammonia and ammonium serve as the primary form of available nitrogen in yeast metabolism in grape juice (Bell & Henchke, 2005; Fugelsang & Edwards, 2007), either by conversion of a non-preferred nitrogen source to ammonia or by growth on ammonia itself (Grenson et al., 1974). The inorganic nitrogen can be fixed into organic forms through reaction with α-ketoglutarate to yield glutamate by glutamate dehydrogenase (**Figure 2-2**). Glutamate can be further used by the cell to produce other amino acids important for metabolism. As part of the metabolism, degradation of nitrogen-containing compounds as the

sole source of nitrogen for yeast growth, leads to the formation of two key nitrogen compounds in yeast cell, ammonium and L-glutamate as summarized by Grenson et al. (1974) and Large (1986). The reactions outlined in **Figure 2-2** show the synthesis of glutamate from α-ketoglutarate and ammonia and the synthesis of glutamine from glutamate and ammonia. By using different sets of enzymes, these core reactions also allow glutamine as the sole source of nitrogen to be converted into glutamate, and for glutamate as the sole source of nitrogen to be converted into glutamine (Magasanik & Kaiser, 2002).

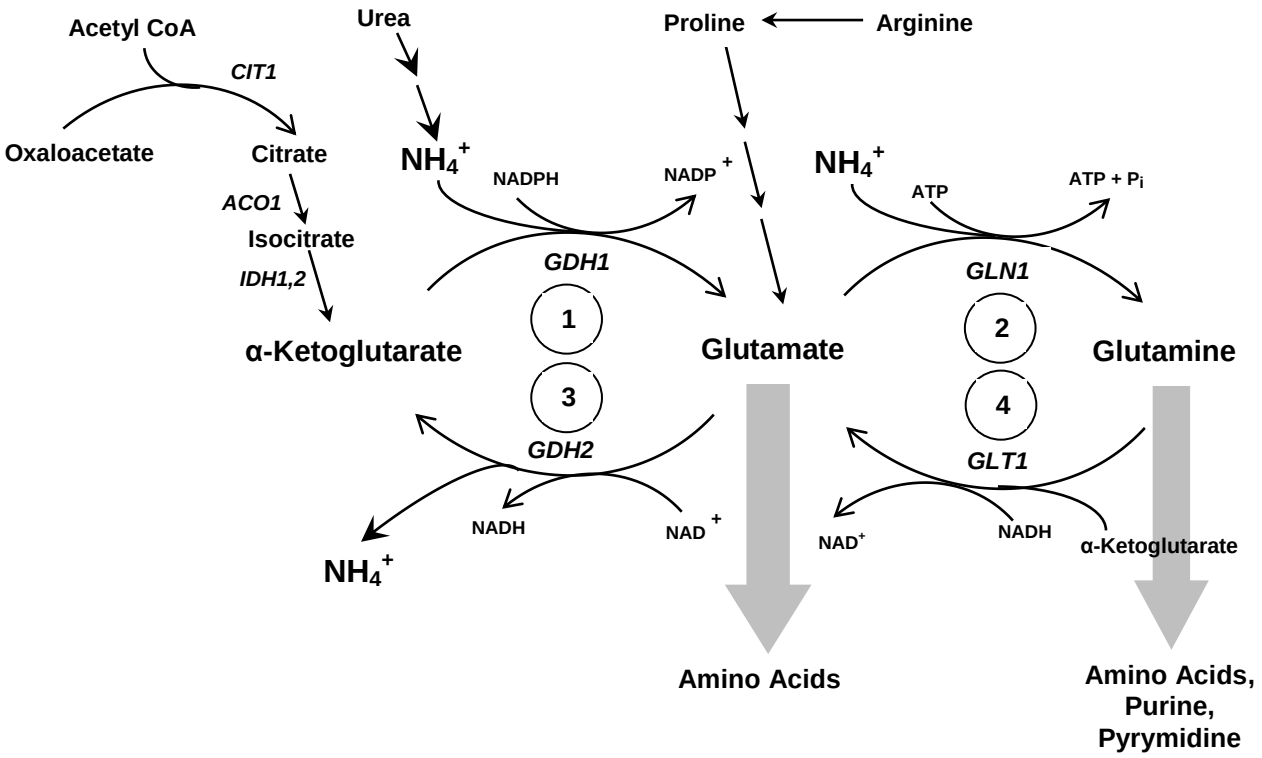


Figure 2-2 Central pathways for nitrogen metabolism
Source: Adapted from Magasanik & Kaiser (2002) and modified

Figure 2-2 showed that the nitrogenous compounds in the cell are synthesized from either glutamate or glutamine. The major pathway for glutamate synthesis is through combination of ammonia with α-ketoglutarate, which is synthesized from acetyl CoA and oxaloacetate through the early steps of the citric acid cycle. Glutamine is synthesized by the combination of ammonia with glutamate. The pathways for utilization of a variety of nitrogen sources, including urea, proline and arginine, are also shown.

The metabolism of nitrogen-containing compounds, α-amino acids, can also make up a major group of wine aroma compounds. Several studies have indicated that both the total available nitrogen and the balance of amino acids and ammonia can significantly affect the production of fermentation-derived volatile compounds, such as higher alcohols or fusel alcohol. They can be produced either by the catabolic conversion of the branched-chain amino acids (valine, leucine, isoleucine, methionine, and phenylalanine) via the Ehrlich pathway or by the anabolic formation of these amino acids de novo from a sugar substrate as shown in **Figure 2-3** (Äyräpää, 1971; Bell & Henschke, 2005; Ugliano et al., 2007; Hazelwood et al., 2008; Ugliano & Henschke, 2009). Higher alcohols are characterized by fusel-like odours, and generally thought to contribute to the complexity of wine fermentation bouquet. However, when present in very high concentrations they can have a negative impact on wine aroma, mainly because they mask fruity characters and can result in a strong, pungent smell and taste (Lambrechts & Pretorius, 2000; Swiegers & Pretorius, 2005; Ugliano et al., 2007). These alcohols together with acids form esters such as acetic acid ethyl ester, acetic acid hexyl ester, acetic acid isoamyl ester (banana-like aroma), hexanoic acid ethyl ester, octanoic acid ethyl ester (apple-like aroma), and acetic acid 2-phenylethyl ester (fruity and flowery like aroma) during fermentation, which significantly contribute to the pleasant fruity aroma of wines and other alcoholic beverages. At low levels, all these compounds contribute to perceived wine aroma complexity. However, they have also been considered as responsible for off-flavours when present in too high amounts (Romano et al., 2003; Bell & Henschke, 2005; Swiegers & Pretorius, 2005; Mendes-Ferreira et al., 2009). Among other factors, nitrogen content affects the pattern of both higher alcohols and esters formed during fermentation, via regulation of the Erhlich, fatty acid, and ester synthesis pathways (Bell & Henschke, 2005; Swiegers & Pretorius, 2005; Mendes-Ferreira et al., 2009)

However, nitrogen metabolism is depending on many factors. For example, temperature, nitrogen deficiency in grape must and timing of nitrogen addition influence the quantity and the quality of yeast nitrogen requirements (Beltran et al., 2005, 2007, 2008). *S. cerevisiae* selects nitrogen sources that enable the best growth by the NCR pathway (Magasanik & Kaiser, 2002; Bell & Henschke, 2005). Good nitrogen sources such as glutamine, asparagine or ammonium decrease the level of enzymes required for utilization of poorer nitrogen sources (ter Schure et al., 2000; Beltran et al., 2004). In addition, utilization of nitrogen-containing compounds by yeasts is depended on not only the yeast strain, its physiological state and the physicochemical properties of the must or wine, but also the fermentation conditions, e.g. yeasts consume less nitrogen at low temperatures and ethanol inhibits the uptake of most amino acids (Bisson, 1991; Valero et al., 2003; Beltran et al., 2007).

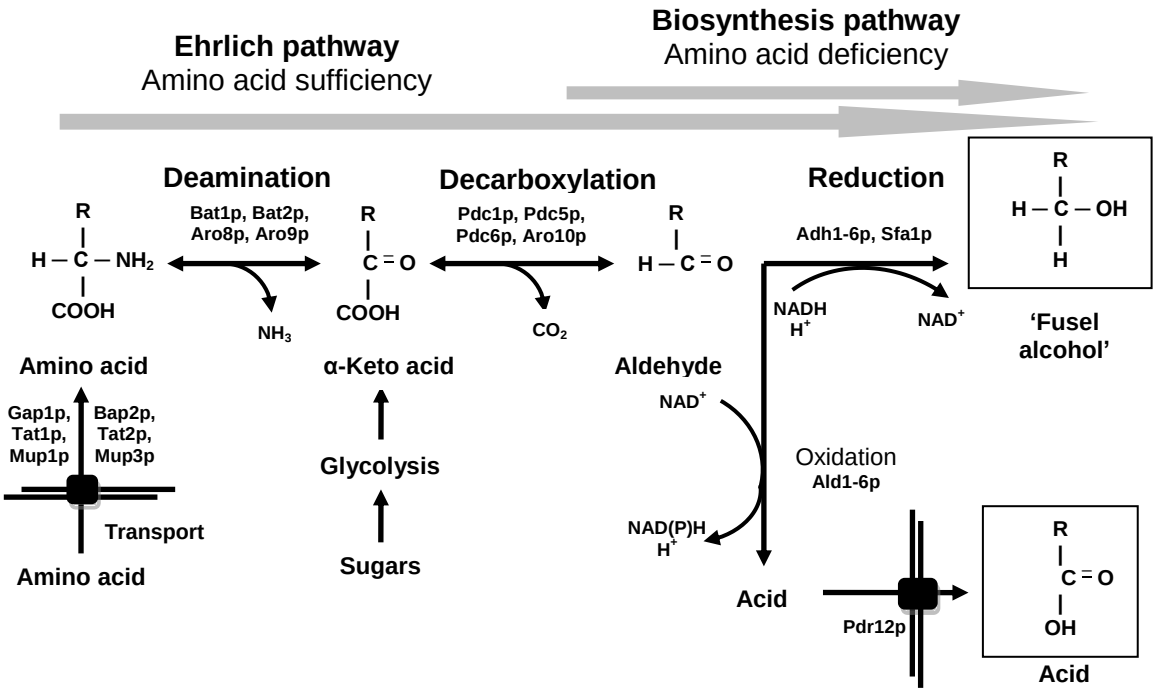


Figure 2-3 Formation of higher alcohols from sugar and amino acids by the Ehrlich pathway
Source: Adapted from Bell & Henschke (2005) and Ugliano & Henschke (2009) and modified

2.3 Odour-active compounds involved in varietal aromas in grape and wines

During the alcoholic fermentation, yeasts do not only convert sugars to ethanol and carbon dioxide, they also produce and release a range of minor but sensorially important volatile metabolites and aromas that give wine its vinous character, however there are diverse mechanisms involved in the development of the aroma compounds. To date, over 700 aroma compounds have been identified, an indication of the potential complexity of wine at concentrations ranging from mg L^{-1} to a few ng L^{-1} or even less (Guth, 1998; Rapp, 1988, 1998; Lambrechts & Pretorius, 2000; Romano et al., 2003; Swiegers et al., 2005 a, 2005 b; Ribéreau-Gayon et al., 2006 b; Fisher, 2007). Consequently, the olfactory impact of the wine aroma depends on the concentration, type and perception value. Furthermore, the impact of each component on the attractiveness of the wine aroma depends on its specific properties. The odouriferous compounds from grapes play a more decisive role in the quality and regional character of wines than any other aroma components. These compounds are responsible for the varietal aroma of wines (Ribéreau-Gayon et al., 2006 b).

The varietal aromas are formed by grape metabolism and are depending on the grape variety, soil, climate and vineyard management techniques. The glyco-conjugates and S-cysteine-conjugates, upon hydrolysis by yeast enzymes, can strongly contribute to wine varietal character (Strauss et al., 1986, Francis et al., 1992, 1996; Francis & Newton, 2005, Swiegers et al. 2005 a, 2005 b; Thibon et al., 2008 b; Rauhut, 2009). Examples of grape-

derived aroma compounds include hexyl derivatives that elicit fruity and floral aromas and some monoterpenes, norisoprenoids, substituted methoxypyrazines and thiols as cysteine conjugates as well as fatty acids, carotenoids and phenolic acids which are enzymatically cleaved to powerful odour compounds, such as 3-*cis*-hexanol, β-damascenone or 4-vinylguaiacol, 4MSP, 3SH. The majority of varietal aroma compounds are present in bound form, making them non-volatile and hence they have no odour. However, these bound compounds can be released or derived during alcoholic fermentation due to bioconservation of yeasts (Cole & Noble, 2003; Swiegers & Pretorius, 2005; Swiegers et al., 2005 a, 2005 b; Fisher, 2007; Rauhut, 2009). Only a few compact substances, such as the monoterpene linalool or the methoxypyrazines, are also present in their free form in the grape and in the juice after pressing. The other source for varietal aroma is acid-catalyzed rearrangements of odourless or barely volatile compounds yielding highly active odourants, such as *cis*-rose oxide or *trans*-rose oxide of Gewürztraminer or 1,1,6-trimethyl-1,2-dihydronaphthaline of aged Riesling wines (Fisher, 2007). Regarding the variety of varietal aroma compounds, only some varietal aromas like monoterpenes and thiols with a pleasant odour are given in this review. The odour-active compounds involved in varietal aroma of wines are shown in **Table 2-4**.

Monoterpenes

The monoterpenes are a diverse class of natural products, which are genuine compounds in grape berries (*Vitis vinifera*) that contribute important floral and citrus characters to wines. Usually the concentrations are below the thresholds, but sometimes in some varieties monoterpenes may contribute to the varietal bouquet. They are regarded as key impact compounds of several white wine varieties, such as Muscat of Alexandria, Morio Muscat, Riesling, Scheurebe and Gewürztraminer, as well as are responsible for their characteristic floral aroma (Mateo & Jiménez, 2000; Ebeler & Thorngate, 2009). Monoterpenes can be divided into three different classes. The first class consists of the free aroma compounds, commonly dominated by linalool, geraniol and nerol, together with the pyran and furan forms of the linalool oxides. However, depending on how the juice has been treated and on factors, which may include climate, many additional monoterpenes can be found in this group, e.g. citronellol, α-terpineol, ho-trienol, nerol oxide, myrcenol, the ocimenols plus several other oxides, aldehydes and hydrocarbons. In wines, several monoterpene ethyl ethers and acetic acid esters have also been found among the free aroma compounds (Mateo & Jiménez, 2000). Secondly, there are the polyhydroxylated forms of the monoterpenes, or free odourless polyols. The most significant features are the polyols. Although these original compounds do not contribute to the aroma, some of them are reactive and can break down with great ease to give pleasant and potent volatiles such as diendiol (3,7-dimethylocta-1,5-

diene-3,7-diol), which can give ho-trienol and nerol oxide (Williams et al., 1980; Mateo & Jiménez, 2000). Finally, these monoterpenes are the glycosidally conjugated forms. While in their glycoside forms, they offer no contribution to the wine's flavour. However, when these compounds are hydrolyzed back to the sugar and corresponding alcohol, it is this process that helps to contribute to the wine's flavour. Fisher (2007) reported that the release from these glycosidic precursors can be achieved by acidic hydrolysis at low pH or by the enzymatic hydrolysis, especially pectinase enzyme as well as yeast strain exhibiting a specific β-glucosidase side activity. Glycosides are, in most cases, more abundant than the unglycosilated forms of individual monoterpenes and polyols (Mateo & Jiménez, 2000). Improvements in separations of these compounds obtained with high-resolution capillary GC columns in the 1970s and 1980s enabled the identification of over 50 monoterpenes in grapes and wines, and the terpene composition is widely used for varietal characterization (Schreier, 1979; Marais, 1983; Rapp, 1988, 1998).

In most cases, the most important compounds are the monoterpene diols, linalool, geraniol, nerol, citronellol, ho-trienol and α-terpineol (Strauss et al., 1986; Guth, 1997 a, 1997 b; Fisher, 2007). Geraniol has an aroma described as rose-like, linalool's aroma is described as camphorous and nerol oxides as vegetative (Simpson, 1979). The flavour threshold of nerol and α-terpineol is three to four times higher than that of linalool (100 µg L^{-1}). The linalool oxides have flavour thresholds of 3000–5000 µg L^{-1} (Rapp, 1988). Besides these compounds, several highly odouriferous cyclic ethers and lactones have been identified as key compounds that are generated by cyclization of oxygenation products from these monoterpene alcohols (Luan et al., 2004). They exist in berry, largely in the skins, principally as glycoconjugates with only a small proportion present in the free form and are liberated during alcoholic fermentation. Monoterpenes exist in grape juice and must principally as mono- and disaccharide terpenes and are released by acidic hydrolysis and various glycosidic enzymes of grape and exogenous origin, such as commercial enzyme preparations added during the wine making process (Strauss et al., 1986). However, acidic conditions present during alcoholic fermentation and storage can catalyze many monoterpene rearrangements, yielding new compounds with different aroma quantities and intensities (Rapp, 1988; Ebeler & Thorngate, 2009). For example, Rapp (1988) investigated that linalool, which is an important floral aroma component of Muscat and Riesling varieties, can be transformed to α-terpineol, hydroxylinalool, geraniol, or nerol under aqueous and acidic conditions. In addition, Da Porto & Battistutta (1995) investigated that the concentrations of glycosidically bound linalool, geraniol, nerol and of free geraniol and β-citronellol can be highly correlated with maceration time.

Table 2-4 The odour-active compounds involved in the varietal aromas in wine

Varietal aromas	Concentration in wine ($\mu g\ L^{-1}$)	Aroma threshold ($\mu g\ L^{-1}$)	Aroma descriptor
Monoterpenes			
cis-Rose oxide	3 - 21	0.2	green, grassy, lychee, rose
Citronella	traces - 12	18	Citronella
Geraniol	0.91 - 506	30, 130	rose blossom, geranium
ho-Trienol	traces - 127	110	Linden
Linalool	1.7 - 473	25.2, 50	flower, lavender, rose, lychee
Nerol	4 - 135	400	Rose
α-Terpineol	3 - 87	400	lily of the valley
Wine lactone	traces - 0.09	0.01	coconut-like, spice (lime)
C13-norisoprenoids			
β-Damascenone	traces - 11.9	0.05	apple, rose, honey, lemon balm
β-Ionone	0.032 - 1.95	0.09	violet, flower, raspberry, seaweed
Vitispriran (E)-6-methylene-2, 10,10-trimethyl-1-oxaspiro [4.5]dec-7-en	20 -320 > 800 in port wine	800	balsamic, resinous
1,1,6-Trimethyl-1,2-dihydronapthalene	1 - 59	20	petroleum-like, kerosene-like
Methoxypyrazines			
3-Isobutyl-2-methoxypyrazine	traces - 0.042	0.002 in water	green bell pepper, spice, earth
3-Isopropyl-2-methoxypyrazine	0.035	0.002 in water	green bell pepper, earth, raw potato, musty
3-*sec*-Butyl-2-methoxypyrazine	0.0005	0.001 in water	green bell pepper

Source: Francis & Newton (2005), Ribéreau-Gayon et al. (2006 b), Fisher (2007)

More recently, several studies suggest that fermentation yeasts might play a significant role in the monoterpene content of wine (Charoenchai et al., 1997; Bell & Henschke, 2005; Carrau et al., 2005; Loscos et al., 2007; Ugliano & Henschke, 2009). In addition to β-glucosidase activities, glycosidic activities capable of hydrolysing disaccharide monoterpene conjugates have also been identified in some fermentations and wine microorganisms (Bell & Henschke, 2005). According to the release of volatile compounds by hydrolysis of non-volatile glycoconjugates, various yeast species associated with fermentation can produce monoterpenes from sugar metabolism, albeit at a low concentration (Carrau et al., 2005). This group also revealed that some wine strains of *Saccharomyces cerevisiae* are capable of

significant production under certain conditions. *Saccharomyces* yeast can produce citronellol from geraniol and nerol, the intensity of this transformation depends on the yeast strain used (Dugelay et al., 1992; Fernández-González et al., 2003; Fernández-González & Di Stefano, 2004). Other authors propose a more complex scheme that geraniol was transformed by these yeasts into geranyl acetate, citronellyl acetate and citronellol, while nerol was transformed into neryl acetate; in addition, geraniol was transformed into linalool and nerol was cyclized to α-terpineol at must pH (Di Stefano et al., 1992). Additionally, the YAN and oxygen content of the fermentation medium influence monoterpene formation, that high YAN (400 mg N L^{-1}) stimulates monoterpene formation, except sesquiterpene (nerolidol and farnesol). Bell & Henschke (2005) summarized that some strains of yeast, especially *Saccharomyces*, might contribute to the floral aroma of wine by *de novo* synthesis of monoterpenes, and this effect could be augmented by higher juice nitrogen in combination with microaerobic fermentation.

Thiols involved in varietal flavour of wines

Thiol compounds (referring to the –SH functional group, the so-called mercaptan) are generally viewed as being responsible for a range of off-flavours (Goniak & Noble, 1987; Rauhut et al., 1995; Ribéreau-Gayon et al., 2006 b; Rauhut, 2009). However, their major contribute to the aroma of certain fruits and aromatic plants has been clearly established. Thus specific thiols are involved in the characteristic aromas of fruit such as blackcurrant (Rigaud et al., 1986; Píry et al., 1995), grapefruit (Demole et al., 1982; Lehmann et al., 1994), passion fruit (Engel & Tressl, 1991; Weber et al., 1994, 1995; Tominaga & Dubourdieu, 2000) and guava (Idstein & Schreier, 1985; Steinhaus et al., 2008).

The first volatile thiol identified in Sauvignon blanc wines was 4MSP (**Figure 2-4**), which elicits aromas like blackcurrant, box tree and broom. Its perception threshold is very low (0.1-0.8 ng L^{-1} in water and model solution) (Darriet et al., 1991, 1993, 1995; Murat et al., 2001 a; Howell et al., 2004). It is always present in Sauvignon blanc at concentrations higher than the threshold (up to 120 ng L^{-1}). However, high concentrations of 4MSP may cause a “catty” note in wines (Tominaga et al., 1998 a, 1998 b). Several other odouriferous volatile thiols (**Figure 2-4**), e.g. 3SH, 3SHA, 3-methyl-3-sulphanylbutan-1-ol (3MSB) and 4MSPOH have also been identified as major contributors to the varietal aromas of Sauvignon blanc wines. The aroma of 3SH is redolent of grapefruit and passion fruit as well as reminiscent of other tropical fruit flavours, such as citrus zest, lychee and guava. The perception threshold is on the order of 60 ng L^{-1} and always present in Sauvignon blanc wine at concentrations of several hundred ng L^{-1}, and there may be as much as a few μg L^{-1}. The complex odour of 3MSHA is reminiscent of boxwood, as well as grape fruit zest and passion fruit. It has a similar threshold value like 4MSP at 4 ng L^{-1} and some Sauvignon blanc wines may contain several

hundred of ng L^{-1}. Nevertheless, its concentrations decrease as the wine ages and 3SH is formed (Darriet et al., 1995; Tominaga et al., 1998 a, 1998 b; Murat et al., 2001 a; Dubourdieu et al., 2006; Ribéreau-Gayon et al., 2006 b). The thiols of 3SH and 3SHA were previously identified in passion fruit by other research group as well (Engel & Tressl, 1991; Weber et al., 1994, 1995; Tominaga & Dubourdieu, 2000).

The aroma of 4MSPOH, which is reminiscent of citrus zest, has a more limited organoleptic role in wines because its concentrations in wine are rarely over the perception threshold (55 ng L^{-1}). However, its threshold can be reached in a few wines. The less odouriferous 3-methyl-3-sulphanylbutan-1-ol (3MSB), which smells like cooked leeks, never reaches the perception threshold of 1500 ng L^{-1} in wine (Dubourdieu et al., 2006; Ribéreau-Gayon et al., 2006 b). In addition, 4MSP, 3SH, 3SHA and other related thiols have also been identified in wines made from other varieties like Merlot, Cabernet Sauvignon (Murat et al., 2001 b), Petite Arvine, Chenin blanc, Bacchus (Ribéreau-Gayon et al., 2006 b), Gewürztraminer, Pinot gris, Riesling, Alsace Muscat, Sylvaner, Pinot blanc, Colombard, Semillon, Petit and Grand Manseng (Tominaga et al., 2000), Scheurebe (Guth, 1997 a, 1997 b) in varying concentrations and can, therefore, have a potential impact on the aroma. In Scheurebe wine, 4MSP was found to be the most potent aroma of 42 isolated odourants analysed. When each of the 42 odourants were individually excluded from a model wine, the absence of 4MSP produced a wine least like the original. Recently, Sarrazin et al. (2007) indicated a contribution of the identified and quantified thiols, 2-methyl-3-sulphanylbutan-1-ol, 3-sulphanylpentan-1-ol, 3-sulphanylheptan-1-ol and probably 2-methyl-3-sulphanylpentan-1-ol, to the overall aroma of sweet wines made from *Botrytis*-infected grapes. **Table 2-5** specifies the organoleptic roles of these volatile thiols found in wines.

In contrast to many tropical fruits, the volatile thiols are not present in their free and odourous form in the grape berries, like their odourless cysteine conjugate (**Figure 2-5**), therefore it has been proposed that the wine yeast, *Saccharomyces cerevisiae*, is responsible for the liberation of volatile thiols from the precursors (Darriet et al., 1993, 1995; Tominaga et al., 1998 c). The precursors to Sauvignon blanc aroma compounds were identified at the Bordeaux Faculty of Enology in the 1990s. Darriet et al. (1993) demonstrated that 4MSP was released from an odourless must extract, either because of bioconversion by yeast during alcoholic fermentation or chemically, in vitro, because of the action of ascorbic acid. Tominaga et al. (1995, 1998 c) also identified odourless sulphur-cysteine conjugates as precursors for the high odour-active thiols.

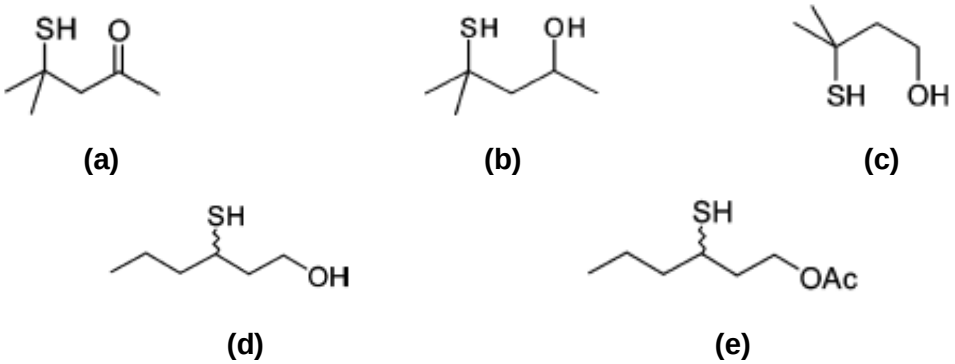
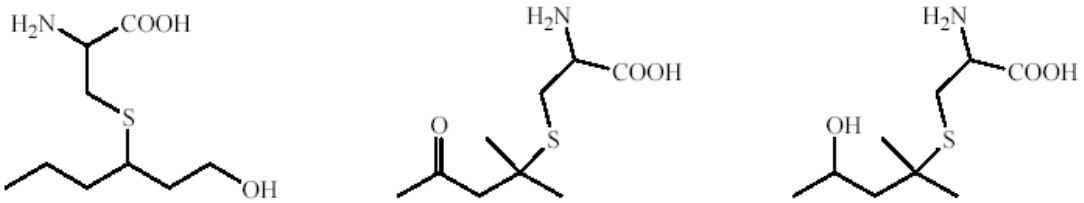


Figure 2-4 Odour-active volatile thiols identified in Sauvignon blanc wine
(a) 4MSP, (b) 4MSPOH, (c) 3MSB, (d) 3SH, (e) 3SHA
Source: Adapted from Vermeulen et al. (2003), Ribéreau-Gayon et al. (2006 b) and Grant-Preece et al. (2010) and modified

Some studies have enabled the identification of *S*-3-(hexan-1-ol)-glutathione for the first time in juice of Sauvignon blanc. The identification of this compound suggests that the *S*-3-(hexan-1-ol)-L-cysteine, the precursor of 3SH in grapes, is produced by the catabolism of *S*-3-(hexan-1-ol)-glutathione (Peyrot des Gachons et al., 2000, 2002 a, 2002 b; Peyrot des Gachons, 2002). It is assumed that *S*-glutathione transferase is involved in the synthesis of the sulphur–glutathione-conjugates, which are probably transported with the help of a glutathione-conjugate-pump to the cell vacuole. The sulphur–cysteine-conjugates are almost certainly formed through the activity of a γ-glutamyl transpeptidase, which removes glutamic acid, and a carboxypeptidase, which eliminates glycine (Wüst, 2003). Tominaga & Dubourdieu (2000) suggested that the volatile thiol of 3SH in passion fruit juice is present in both free and conjugate form and have also identifed the precursor of 3SH as *S*-(3-hexan-1-ol)-L-cysteine, in the form of trimethylsilylated derivatives in passion fruit. These cysteine conjugates are probably converted into free thiols either by acid hydrolysis, or by an endogenous enzyme, such as a β-lyase.

The 4MSP and 4MSPOH precursors are mainly located in the flesh of berry (approximately 80 %), while the skin and flesh contain equal amounts of 3SH precursor (Peyrot des Gachons et al., 2002 a). Similarly, Murat et al. (2001 b) investigated that a majority (60 %) of the 3SH precursor is located in the skins of Cabernet Sauvignon and Merlot grapes. In the production of rosé wines made from Cabernet Sauvignon, Cabernet Franc and Merlot, it was shown that increased skin contact time (from 0 to 24 hours) correlated with a higher extraction of the 3SH precursor, as well as an increased concentration of volatile 3SH following yeast fermentation. Additionally, this phenomenon was more marked at higher temperatures. In the vinification of Sauvignon blanc, skin contact for 19 hours also increased

the concentration of the precursor in the pressed juice, e.g. Cys-4MSPOH content has increased by 20 %, Cys-4MSP by 30 % and Cys-3SH by 50 % (Peyrot des Gachons, 2002). Regardless of the skin contact temperature, maceration only slightly increases the concentrations of Cys-4MSPOH and Cys-4MSP with respect to a non-macerated must (pressed immediately off its skins). In addition, low maceration temperature limits the increase in Cys-3SH, probably because it decreases extraction from the solids.



S-3-(hexan-1-ol)-L-cysteine (Cys-3SH)

S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4MSP)

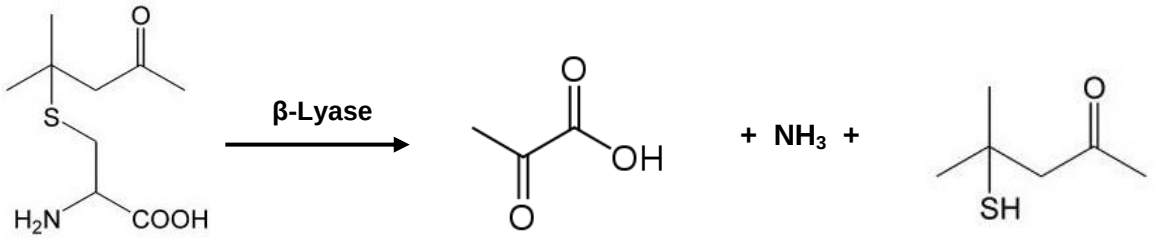
S-4-(4-methylpentan-2-ol)-L-cysteine (Cys-4MSPOH)

Figure 2-5 The sulphur-cysteine conjugates

Source: Adapted from https://people.ok.ubc.ca/neggers/Chem422A/VARIETAL%20AROMA%20COMPOUNDS.pdf and modified

The release of the volatile thiols was investigated by the use of a cell-free enzyme extract of the bacterium *Eubacterium limosum* that contains carbon-sulphur lyase enzymes. It could be shown that carbon-sulphur lyase enzymes can release 4MSP and 4MSPOH from their precursor *S*-4-(4-methlypentan-2-one)-L-cysteine (Cys-4MSP) and *S*-4-(4-methylpentan-2-ol)-L-cysteine, respectively. Therefore, it was suggested that a yeast cysteine β-lyase releases a thiol, pyruvate and ammonium from the corresponding sulphur-cysteine conjugates as shown in **Figure 2-6** (Tominaga et al., 1995; Peyrot des Gachons et al., 2000, 2002 a, 2002 b; Peyrot des Gachons, 2002; Wakabayashi, 2004). Although the extraction of the cysteine conjugated precursors into the juice appears to be correlated to the final concentrations of the volatile thiols present in the wine, only a small and varying proportion of the precursor is converted to the active aroma compound during fermentation. Where this release has been studied, only 5 % of the potential 4MSP was released during fermentation (Tominaga et al., 1995, 1998 c). Peyrot des Gachons (2002) also found that the percentages of transformation (4MSP and 4MSPOH precursors into 4MSP and 4MSPOH aroma compounds) are very low, e.g. mean values are 1.4 % for Cys-4MSP and 3.0 % for Cys-4MSPOH. Studies of the formation of the free thiol have shown that a bacterial extract from *Eubacterium limosum* or purified tryptophanase from *Escherichia coli* cleaves the Cys-3SH precursor in vitro (Tominaga et al., 1998 c; Wakabayashi, 2004; Wakabayashi et al., 2004).

The bacteria extracts exhibit cysteine-*S*-conjugate β-lyase activity, therefore the wine yeast *Saccharomyces cerevisiae* is proposed to cleave thiol precursors during grape juice fermentation and to release 3SH by a similar mechanism (**Figure 2-7**) (Tominaga et al., 1998 c). It has been shown that β-lyase enzyme activity results in the formation of a free thiol and an intermediate that spontaneously degrades to pyruvate and ammonia (Davis & Metzler, 1972). It has been shown that when the thiol precursor of Cys-3SH decreases in concentration, 3SH increases during fermentation (Murat et al., 2001 a), however, 0–9 % of 3SH was released over the course of fermentation. Furthermore, in Cabernet Sauvignon and Merlot musts, it was shown that the amount of 3SH released was proportional to the Cys-3SH concentration present at the start of fermentation, although only 3.2 % of the original precursor originally was released as volatile thiols in the must. Therefore, the higher the concentration of the cysteine conjugate thiol precursors in the must, the higher the volatile thiol concentration in the resulting wine (Murat et al., 2001 a, 2001 b). It has been confirmed by Peyrot des Gachons (2002) that the percentage of transformation of 3SH precursor (Cys-3SH) into 3SH aroma compound is very low, only 4.2 % of 3SH was released. Similarly, Dubourdieu et al. (2006) also reported that only a small fraction (1.6 %) of the cysteine-bound precursor originally present was released as 3SH.

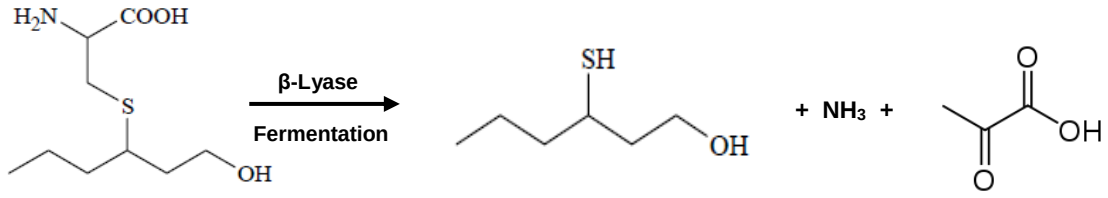


S-4-(4-methylpentan-2-one)-L-cysteine **Pyruvate** **4-Methyl-4-sulphanylpentan-2-one**

Figure 2-6 Release of 4-MSP from the corresponding sulphur–cysteine-conjugate
Source: Adapted from Rauhut (2009) and modified

It has been shown that, during fermentation, 3SHA is generally formed when acetic acid esterifies the 3SH that has been released. Swiegers et al. (2005 b, 2007) investigated that 3SHA is formed from 3SH by the action of the yeast ester-forming alcohol acetyltransferase, encoded by the *ATF1* gene. The overexpression of the *ATF1* gene in the VIN13 yeast strain resulted in a significant increase in the amount of 3SHA produced. This established the link between ester production and volatile thiol metabolism in yeast for the first time. On the other hand, overexpression of the gene *IAH1*, which encodes an ester-degrading enzyme, resulted in a reduction in the concentration of 3SHA. The ability of different commercial wine yeasts to convert 3SH into 3SHA during fermentation was also investigated. Large variations in 3SHA

concentrations were observed, and in most cases, this did not correspond with the ability of the yeasts to release 4MSP. Therefore, it is clear that yeast strain selection is of extreme importance in modulating volatile thiol-concentrations in wine. Lilly et al. (2006) also suggested that the overexpression of *ATF1* in a VIN13 yeast strain resulted in increased 3SHA concentrations.



S-3-(hexan-1-ol)-L-cysteine **3-Sulphanylhexanol** **Pyruvate**

Figure 2-7 The cysteine conjugate form of 3SH, which revealed by a specific β-lyase
Source: Adapted from https://people.ok.ubc.ca/neggers/Chem422A/VARIETAL%20AROMA%20COMPOUNDS. pdf and modified

Recently, Roland et al. (2010) suggested that the production of the 3SH in wines seems to have two different origins, the first one from precursors naturally occurring in grapes and the second one linked to the winemaking technology (hexenal and G3SH pathways). The hexenal pathway described by Schneider et al. (2006) implicated sulphur donors during winemaking, and glutathione seemed to be one of the producing 3-*S*-glutathionylhexan-1-ol (Glut-3SH) precursor. They demonstrated a new pathway leading to 3SH and 4MSP, starting from conjugated carbonyl compounds, alternative to the already biogenetic route known release from cysteinylated and gluthationylated precursors present in grapes. It was the first demonstration that Glut-3SH can liberate 3SH under model fermentation conditions, where the cysteine conjugate is also formed in the process. Therefore, it was suggested that this might also occur during fermentation of a grape juice or must (Grant-Preece et al., 2010). It was also investigated in the fermentation of Sauvignon blanc that the additions of glutathione or hexenal induced significant increases in the production of 3SH and 3SHA of 25 % and 41 %, respectively (Roland et al., 2010).

Some research works indicated that yeast strains vary in the ability to release 4MSP, 3SH and 4MSPOH. It could be also demonstrated that commercial yeast strains differ in the release of the various volatile thiols (Murat et al., 2001 a; Howell et al., 2004; Curtin et al., 2009; Swiegers et al., 2005 b, 2007, 2009). Therefore, the genetic and physiological characteristics of the wine yeast strain have a significant effect on the amount of volatile thiols released. In addition, the particular yeast's ability to release one thiol does not appear

to be linked with its ability to release a second, different thiol. For example, the commercially available *S. cerevisiae* wine strains VL3 and EG8 release more 4MSP and 4MSPOH, but not 3SH, than strains VL1 and 522d. It has been shown that strains of *S. bayanus* and their hybrids created with *S. cerevisiae* released even higher concentrations of the thiols (Murat et al., 2001 a). Similarly, Masneuf-Pomarède et al. (2002) investigated that *S. bayanus* var. *uvarum* strains and hybrids *S. cerevisiae* x *S. bayanus* var. *uvarum* were shown to present a high ability to release the volatile thiols from their natural precursors. These findings were recently confirmed by showing that different commercial yeast strains have variable abilities in releasing 4MSP from the Cys-4MSP precursor in model ferments (Howell et al., 2004). Commercial wine yeast strains that release even more thiols than VL3 were recently identified by Swiegers et al. (2005 b), e.g. the VIN7 strain produced the highest concentration of 4MSP and 3MSA and the VIN13 strain produced the highest concentration of 3SH. Therefore, these results indicate that the activity of the enzymes involved in the release of the different thiols is strain dependent and that by using different strains. The variation of the release of the enzymes and of the thiols can be achieved by the use of specific yeast strains. Furthermore, separate yeast enzymes may be involved in the formation of different volatile thiols, allowing the levels of the aroma compounds to be altered independently (Murat et al., 2001 a; Dubourdieu et al., 2006). Post-fermentation practices could also affect the impact of the thiol aromas, for example aroma intensity is reduced when copper is added to Sauvignon blanc wine (Darriet et al., 1995).

In addition, King et al. (2008) investigated the impact of coinoculating commercial yeast strains (VIN7, QA23, VIN13) on the volatile thiols and sensory profile of Sauvignon blanc wines. It was indicated that the VIN7/QA23 coinoculated wines were higher in 3SH and 3SHA than the single-strain and blended wines, although this pattern was not observed for the VIN7/VIN13 yeast combination. This demonstrates that coinoculated wines can result in increased concentrations of the volatile thiols when certain yeast combinations are used to conduct alcoholic fermentation. The ability of yeast to release 4MSP from Cys-4MSP when genes encoding putative yeast carbon-sulphur lyases are deleted was recently investigated. Four genes expressing putative carbon-sulphur lyase enzymes were involved in the release of the volatile thiol 4MSP in a laboratory strain (Howell et al., 2005). This pointed out that the mechanism of release probably involves multiple genes and the result showed that deletion of the four putative carbon-sulphur lyase genes leads to a decrease in the amount of 4MSP released. However, it was not indicated if overexpression of the genes resulted in an increase in 4MSP release. Swiegers et al. (2007) demonstrated that the overexpression of the *Escherichia coli tnaA* gene, encoding a tryptophanase with strong cysteine-β-lyase activity, in a commercial wine yeast strain resulted in up to a 25-fold increased release of 4MSP and 3SH from the precursor in model ferments. In addition, Sauvignon blanc wine

made with this modified yeast displayed an intense passion-fruit aroma, compared with the relatively neutral aromas produced by the control strain.

During the past decade, studies have clearly shown that volatile thiol levels decrease in the presence of oxygen during wine ageing and after bottling (Blanchard et al., 2004; Brajkovich et al., 2005). Aromatic degradation observed in wines is well documented. During aging, an important decrease of 3SH occurs in wine due to the presence of dissolved oxygen. 3SH is probably oxidized to its disulphide (Darriet, 2002). Consequently, this thiol could react with reactive species present in wine such as polyphenols, e.g. the quinones of catechin and epicatechin (Blanchard et al., 2004; Nikolantonaki et al., 2009). In contrast, 4MSP was less reactive with these phenolics. Additionally, the presence of free sulphur dioxide enhances the stabilization of 3SH and 4MSH in model wine. Recently, the impact of fermentation temperature on the concentration of volatile thiols was determined in a model medium and in grape juice. It was shown that the concentrations of 4MSP, 3SH, and 3SHA were higher when the alcoholic fermentation was conducted at 20 °C compared to 13 °C, irrespective of the yeast strain used (Masneuf-Pomarède et al., 2006). In contrast, Swiegers et al. (2006) showed that, in model ferments, more 4MSP was released and more 3SH was converted to 3SHA at lower temperatures (18 °C) compared to higher temperatures (23 and 28 °C) at the end of fermentation. However, at the start of fermentation, more volatile thiols were present in the warmer ferments.

It has been shown that viticultural practices like nitrogen feeds (Choné et al., 2006) and prefermentation operations such as skin contact (Peyrot des Gachons et al., 2002) modulate the amount of thiol precursors in grape must. Fermentation conditions such as temperature (Masneuf-Pomarède et al., 2006; Swiegers et al., 2006) also influence the final concentration of these thiol aromas in wine. The impact of nitrogen levels during fermentation on the release of volatile thiols has not yet been clearly elucidated. There have been little scientific researches about the impact of yeast rehydration nutrient, DYNASTART®, on the release of volatile thiols. Initial results published by Swiegers et al. (2008) revealed that it had a significant effect on both volatile thiol release and fermentation ester production. Similarly, van der Westhuizen et al. (2008) investigated the impact of yeast strains and nutrition on the thiol intensities. It was shown that the rehydration of the X5 yeast strain with yeast rehydration nutrient, DYNASTART, can dramatically increase aromatic intensity of varietal aromas, particularly the 3SHA (passion fruit), even better than a blend of two yeasts commonly expressing high concentrations of volatile thiols. It has been confirmed by Bowyer et al. (2008) that DYNASTART enhanced the expression of varietal aromas in Sauvignon blanc by an average of 30 % for 4MSP, 55 % for 3SH, and 89 % for 3SHA. In addition, Sauvignon blanc wine made with X5 and yeast rehydration nutrient, leaded to significantly higher wine preference.

The research of Subileau et al. (2008) recently investigated the influence of nitrogen catabolite repression (NCR) on the modulation of the production of aromatic thiols characteristic for Sauvignon blanc wine fermentation. It was revealed that the production of 3SH is increased when urea is substituted to diammonium phosphate (DAP) as the sole nitrogen source on a synthetic medium, similarly, on grape must, complementation with DAP induces a decrease of 3SH production. Additionally, it could be concluded that on synthetic medium, Cys-3SH enters the cell through at least one identified transporter, GAP1p, whose activity is limiting the release of volatile thiols. On grape must, the uptake of the precursor through GAP1p is not confirmed, but the effect of addition of DAP, eventually prolonging NCR, is shown to decrease thiol production. More recently, Thibon et al. (2008 a) investigated the role of NCR in the release of volatile thiols in an enological context. The role of three yeast β-lyases was revealed and it was demonstrated that Irc7p, a putative cystathionine (-lyase), is one of the main proteins catalyzing the 4MSP and 3SH release under enological conditions. Moreover, Ure2p/Gln3p proteins mainly control the bioconversion of volatile thiols by the transcriptional regulation of the IRC7 gene through the general mechanism of NCR. In addition, this finding suggested that the enantiomer balance of 3SH may be modulated by activating specifically stereoselective enzymes such as Irc7p.

2.4 Sulphur containing compounds in winemaking

Sulphur (*S*)-containing compounds occur in a range of chemical combinations in the natural environment. Sulphur is present in essential vitamins, coenzymes and amino acids such as methionine and cysteine (Richmond, 1973; Rauhut, 1993). It is important for the growth of all microorganisms due to the formation of sulphur containing amino acids. It can occur in an oxidized state (sulphate) or a reduced form (sulphide). Sulphur is one of the most important elements required for biological life, particularly as a component of the amino acids, cysteine and methionine as well as a component of vital co-factors (Swiegers & Pretorius, 2005; Rauhut, 2009). In *S. cerevisiae*, the sulphur content ranges from 0.2-0.9 % of the dry weight (Maw, 1963 a, 1963 b). The most abundant *S*-compounds are the amino acids methionine and cysteine, which are present in peptides, proteins, tripeptides and glutathione (Hartnell & Spedding, 1979; Rauhut, 1993). Other *S*-compounds, such as thiamine, acetyl-CoA, biotin and lipoic acid, represent only a small proportions of the total sulphur. In *Sacchromyces spp*., thiamine ranges from 29-90 μg g^{-1} dry weight of the cells (Maw, 1965; Rauhut, 1993).

In recent years, the composition in sulphur compounds of wines has become a subject of many studies concerning their identification and origin, as well as their character and impact on wine quality, especially sensory characteristic of wine (Rauhut & Kürbel, 1994; Rauhut, 1996; Moreira et al., 2002; Rauhut, 2003; Fedrizzi et al., 2007; Ferreira et al., 2007; Swiegers

& Pretorius, 2005; Thibon et al., 2008 a, 2008 b; Rauhut, 2009). This is related to their high volatility, reactivity and impact at very low concentrations (Rauhut, 2009). These compounds are generally classified as detrimental to wine quality however, new developments in wine research allowed the differentiation of a family of these compounds responsible for varietal aromas of wines, such as passion fruit, grapefruit, gooseberry, guava, and box hedge aromas in wine. Rauhut (1993, 2009) reported that some of these compounds are necessary for wine quality, while others are the cause of strong objectionable flavours (rotten eggs, cooked cabbage, cauliflower, burnt rubber etc.), even at extremely low concentrations (e.g. H_2S, methanethiol (MeSH), ethanethiol (EtSH), thioacetic acid-*S*-methyl ester (MeSAc), thioacetic acid-*S*-ethyl ester (EtSAc). Several studies reported that during alcoholic fermentation the wine yeast *Saccharomyces cerevisiae* is mainly responsible for the production of various volatile sulphur compounds, which have an impact on the sensory quality of wine (Rauhut, 1993, 1996; Rauhut et al., 1995, 1996; Moreira et al., 2002; Swiegers & Pretorius, 2005; Ribéreau-Gayon et al., 2006 b; Swiegers et al., 2006; Rauhut, 2009). Sulphur compounds in wine can be classified into thiols (mercaptan), sulphides, thioesters and heterocyclic compounds. Furthermore, these compounds can become less or more attractive or repulsive depending on their absolute and relative concentrations. Sulphur aroma compounds in wine were often separated in low and high volatile sulphur substances due to the broad range of different boiling points and the need of different analytical methods to enrich the sulphur compounds from wines (Rauhut et al., 2005 a, 2005 b; Swiegers & Pretorius, 2005; Fedrizzi et al., 2007; Ferreira et al., 2007; Thibon et al., 2008 b).

Most sulphur containing compounds in wine are produced during alcoholic fermentation by the *Saccharomyces* wine yeast (Rauhut, 2009). Volatile sulphur compounds are formed through several pathways involving enzymatic and/or non-enzymatic processes. Yeast fermentation biochemistry with sulphate-, sulphite-, sulphur-containing amino acids (methionine and cysteine) and oligopeptides (e.g. glutathione) plays a crucial role among the enzymatic processes. On the other hand, non-enzymatic processes involve chemical, photochemical and thermal reactions during winemaking and storage. Mestres et al. (2000) suggested that the development of various sulphur compounds by yeast include (i) the degradation of sulphur containing amino acids, (ii) the degradation of sulphur containing pesticides and (iii) the release and/or the metabolism of grape-derived sulphur-containing precursors.

In accordance with the new rules for the international nomenclature of chemical compounds (IUPAC), the prefix ‘methyl-sulphanyl’ should replace the prefix ‘methylthio’ and ‘ethyl-sulphanyl’ should replace the prefix ‘ethylthio’, furthermore the prefix ‘sulphanyl’ must replace the prefix ‘mercapto’ (Rauhut, 2009).

Utilization of sulphur containing compounds by yeasts

Yeast is able to use various sulphur containing compounds in contrast to many other microorganisms due to the sulphur pathway in yeast, which allows it to use various organic and inorganic S-compounds as sole sulphur source (Rauhut, 1993, 2009). A source of assimilable sulphur is essential for yeast growth. Almost all yeasts utilize inorganic sulphate (Maw, 1963 a, 1963 b, 1965). The main sulphur source for yeast during wine making is inorganic sulphur as sulphate, which is presents in grape must in a range of 160-400 mg L^{-1} or even more (Lemperle & Lay, 1989), because the levels of methionine and cysteine are normally very low in grape musts (less than 10 mg L^{-1}) in comparison to other amino acids (Henschke & Jiranek, 1991, 1993). Furthermore, sulphur dioxide and elemental sulphur from residues of the application of wettable sulphur (fungicide) on grape are also the sulphur sources for yeast during the winemaking process. Sulphur dioxide is commonly used due to its antimicrobial and antioxidant effects as well as its reaction with ethanal (acetaldehyde) to avoid an oxidative character and its inactivation of enzymes (Dittrich, 1987; Romano & Suzzi, 1993; Ribéreau-Gayon et al., 2006 a; Rauhut, 2009).

Saccharomyces can metabolize sulphur containing compounds by the sulphate assimilatory reduction pathway or sulphate reduction sequence (SRS) pathway, during which sulphate is taken up and is used for the biosynthesis of organic sulphur compounds, mostly cysteine, methionine, and *S*-adenosylmethionine (AdoMet) (Thomas & Surdin-Kerjan, 1997; Surdin-Kerjan, 2003). Research works to investigate sulphur metabolism in *S. cerevisiae* were done by some research groups, which characterized more than 15 genes encoding enzymes of the sulphur amino acid pathway (Thomas & Surdin-Kerjan, 1997; Henschke & Jiranek, 1993; Surdin-Kerjan, 2003; Wang et al., 2003; Linderholm et al., 2008; Rauhut, 2009). The first step of the SRS metabolic pathway involves the transport of sulphate from the medium into the yeast cell by sulphate permease. Sulphate is then reduced to sulphide through a series of steps using the enzymes ATP-sulphurylase (using two ATP molecules) and sulphite reductase. The next step leads to the sequestering of the sulphide: *O*-acetylserine (from the amino acid serine) combines with sulphide to form cysteine, and *O*-acetylhomoserine (from the amino acid aspartate) combines with sulphide to form homocysteine, which can then be converted to methionine (Yamagata, 1989; Rauhut, 1993; Jiranek et al., 1995 b; Spiropoulos et al., 2000; Henschke & Jiranek, 1993; Rauhut, 2009). **Figure 2-8** shows a simplified overview on the metabolism of sulphur amino acids and glutathione in *Saccharomyces cerevisiae*. Regarding the variety of sulphur containing compounds, only some compounds found in musts and wines are presented in this review.

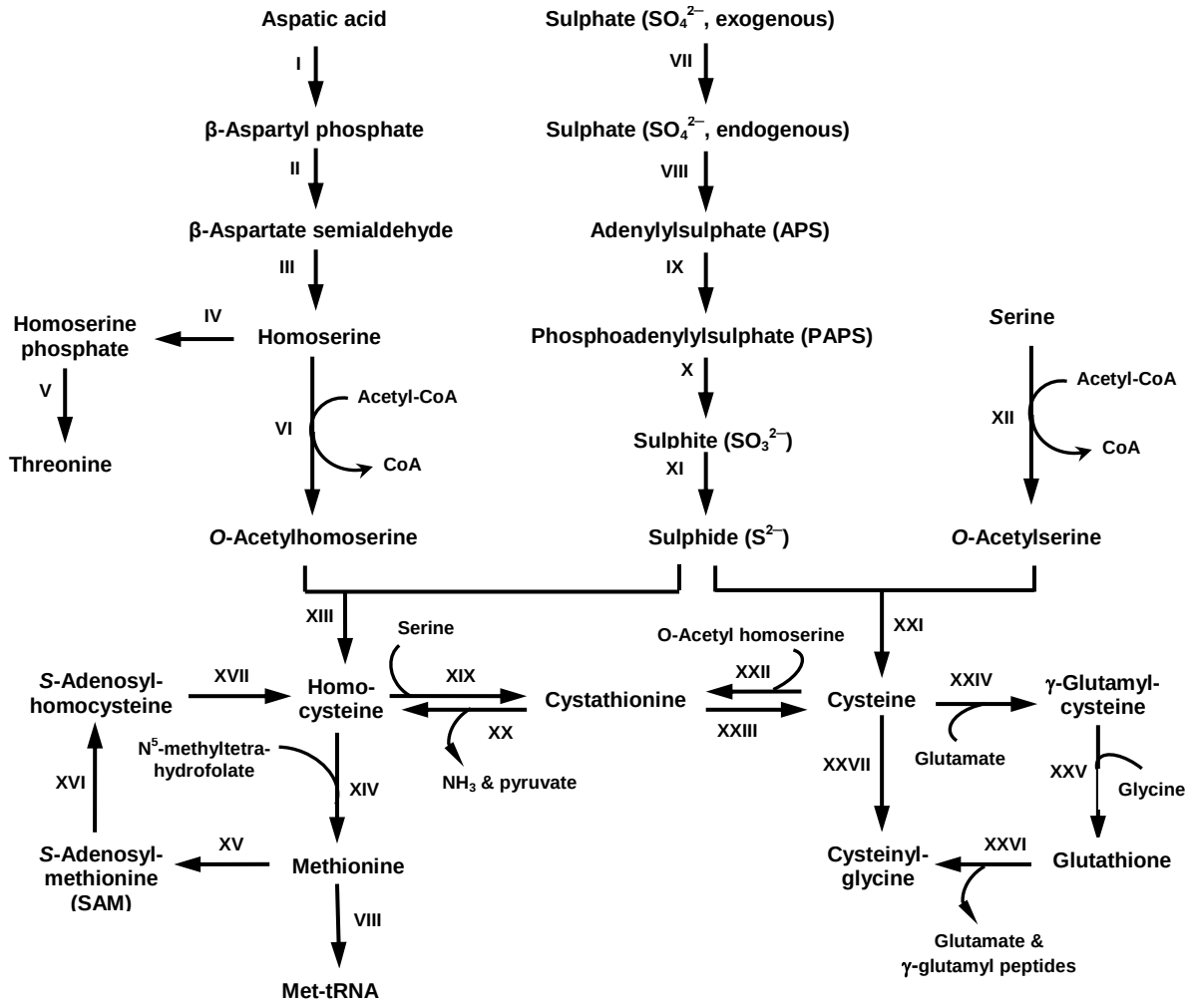


Figure 2-8 Metabolism of sulphur amino acids in *Saccharomyces cervisiae*
Source: Adapted from Spiropoulos et al*.* (2000) and Wang et al. (2003) and modified

(I) aspartate kinase; (II) aspartate semi-aldehyde dehydrogenase; (III) homoserine dehydrogenase; (IV) homoserine kinase; (V) threonine synthase; (VI) homoserine *O*-transacetylase; (VII) sulphate permeases; (VIII) ATP sulphurylase; (IX) APS kinase; (X) PAPS reductase; (XI) sulphite reductase; (XII) serine acetyltransferase; (XIII) *O*-acetylhomoserine and *O*-acetylserine sulphydrylase; (XIV) homocysteine methyltransferase; (XV) *S*-adenosylmethionine synthetase; (XVI) *S*-adensylmethionine demethylase; (XVII) adenosylhomocysteinase; (XVIII) methionyl-tRNA synthetase; (XIX) *β*-cystathionine synthase; (XX) *β*-cystathionase; (XXI) cysteine synthase; (XXII) *γ*-cystathionine synthase; (XXIII) *γ*-cystathionase; (XXIV) *γ*-glutamylcysteine synthetase; (XXV) glutathione synthetase; (XXVI) *γ*-glutamyltranspeptidase; (XXVII) cysteinylglycine dipeptidase

Hydrogen sulphide and related sulphur compounds

During wine fermentation, the assimilatory reduction in sulphate by *Saccharomyces* wine yeast (to biosynthesize cysteine and methionine) can lead to the excessive production of the HS^- ion, which causes the formation of H_2S in wine (Jiranek et al., 1995 b; Spiropoulos et al., 2000; Mendes-Ferreira et al., 2002; Swiegers et al., 2005 a; Mendes-Ferreira et al., 2009). It is generally agreed that H_2S acts as an intermediate in the biosynthesis of sulphur-containing amino acids by yeast during fermentation (Jiranek et al., 1995 b; Thomas & Surdin-Kerjan, 1997; Surdin-Kerjan, 2003), but it is responsible for the "reductive" off-flavours often described as rotten egg and putrefaction in wine (Henschke & Jiranek, 1991, 1993; Rauhut et al., 1995, 1997; Fedrizzi et al., 2007; Rauhut, 2009). Lower levels in young wines contribute to the yeast flavour or fermentation bouquet (Monk, 1986; Dittrich, 1987). The information on the threshold value for this compound is in the range of 11-80 $\mu g\ L^{-1}$ (Amoore & Hautala, 1983; Henschke & Jiranek, 1991). Some studies investigated that deficiency of nitrogen containing nutrients, especially assimilable amino acids, lead to an overproduction of H_2S and other undesirable volatile S-compounds (Rauhut et al., 1995; Rauhut, 1996; Rauhut et al., 2000 a, 2000 b). The concentration of H_2S produced during wine fermentation depends on several environmental factors and on nutrients, namely (1) levels of elemental sulphur (Schütz & Kunkee, 1977; Thomas et al., 1993) naturally available as sulphate at an average concentration of 200 mg L^{-1} (Rauhut, 1993, 2009) and residues from wettable sulpuhr treatment of the vines and other sulphur containing pesticides and their breakdown products (Rauhut, 1993, 2003; Henschke & Jiranek, 1993; Bell & Henschke, 2005; Ribéreau-Gayon et al., 2006 a, 2006 b; Rauhut, 2009), (2) presence of sulphur dioxide (Acree et al., 1972; Romano & Suzzi, 1993; Ribéreau-Gayon et al., 2006 a) commonly added (50-200 mg L^{-1}) to grape must prior to wine fermentation, (3) presence of organic compounds containing sulphur (Henschke & Jiranek, 1991; Giudici & Kunkee, 1994; Rauhut, 2009), (4) wine yeast strain (Jiranek et al., 1995 b; Rauhut et al., 1995, 1996, 1997; Spiropoulos et al., 2000; Mendes-Ferreira et al., 2002, 2009), (5) fermentation conditions, (6) the nutritional status of the grape juice, (7) residues of copper ions and storage on lees (Henschke & Jiranek, 1991; Rauhut, 1993; Rauhut et al., 1997, 2000 a, 2000 b, 2001; Rauhut, 2003; Bell & Henschke, 2005; Mendes-Ferreira et al., 2009) and (8) vitamin deficiency (Wainwright, 1971; Tokuyama et al., 1973; Wang et al., 2003; Bohlscheid et al., 2007; Mendes-Ferreira et al., 2009). However, some strains appear to produce H_2S constitutively without being affected by the environmental conditions, possibly indicating metabolic defects (Jiranek et al., 1995 b; Spiropoulos et al., 2000; Mendes-Ferreira et al., 2002).

Accumulation of acetaldehyde and the elongator histone complex are suggested as two cellular activities that have an impact on sulphide production during anaerobic fermentation (Rauhut, 2009). Recent evidence suggests that intracellular glutathione can be degraded to

cysteine and ultimately H_2S under nitrogen deficit conditions (Elskens et al., 1991, Hallinan et al., 1999), this result is in accordance with Rauhut et al. (2001) and Rauhut (2003), who investigated that the supplementation of must with a concentration of more than 50 mg L^{-1} can lead to unpleasant volatile sulphur compounds under certain conditions. On the contrary, Dubourdieu & Lavigne-Cruège (2002) proposed that glutathione seems to play an important role in protecting volatile thiols that are responsible for the varietal flavour of white wines during the ageing of bottles. H_2S is known to be produced directly from cysteine by cysteine desulfhydrase when nitrogen is limited (Tokuyama et al., 1973). Insufficiencies in vitamins, micronutrients (pantothenic) and vitamin B_6 (pyridoxine), essential for the synthesis of sulphur containing amino acids, may also contribute to H_2S formation (Jiranek et al., 1995 b; Spiropoulos et al. 2000; Mendes-Ferreira et al., 2002). Ammonium salts like diammonium hydrogen phosphate (DAP) are widely used to compensate nitrogen deficiencies in grape must and to practically control H_2S formation (Hansen et al, 1994; Rauhut, 2009). However, not in all cases its supplementation is effecting H_2S production due to other factors, e.g. methionine and other nitrogen sources that regulate amino acid transport into the yeast cell and sulphur metabolism, especially SRS pathway, which generates H_2S (Spiropoulos et al., 2000; Spiropoulos & Bisson, 2000). Some studies have indicated that DAP is a powerful modulator of H_2S and other fermentation-derived volatiles, however, depending on yeast strain (Jiranek et al., 1995 b; Wang et al., 2003; Hernández–Orte et al., 2006 a; Vilanova et al., 2007; Ugliano et al., 2009). Furthermore, the problem of overproduction of H_2S is relatively easily dealt with through the use of copper, which result in the formation of copper sulphide, or aeration resulting in oxidation of the sulphide (Monk, 1986; Rauhut, 2009).

In addition to hydrogen sulphide and related sulphur compounds, a variety of other thiols exist in wine, which are though usually present in only very low concentrations. They generally cause undesirable aromas, however, several confer pleasant fruity aromas (Rauhut, 1993; Darriet et al., 1995; Tominaga et al., 1998 a, 1998 b; Bell & Henschke, 2005; Ribéreau-Gayon et al., 2006 b; Swiegers et al., 2005 b; Rauhut, 2009). Nevertheless, the important varietal thiols are already reviewed in more details in **2.3 Thiols involved in varietal flavour of wines**. Many of these S-compounds are formed during fermentation and respond to the nitrogen status of the must in a similar manner to H_2S (Bell & Henschke, 2005). It has been well known that hydrogen sulphide is a highly reactive substances, which can take part in a range of reactions to generate some sulphur compounds that have an impact on the flavour of a wine, such as ethanethiol (ethyl mercaptan), dimethyl sulphide (DMS) and polysulphides (dimethyl disulphide, DMDS), dimethyl trisulphide (DMTS) and dimethyl tetrasulphide) (Rauhut, 1993; Vermeulen et al., 2003; Bartowsky & Pretorius, 2009; Rauhut, 2009). One mechanism for the formation of the polysulphides is believed to involve oxidation of the mercaptans. These compounds, which elicit a “rubber” or “garlic” odour

cannot be removed by copper fining (Swiegers et al., 2005 a). Goniak & Noble (1987) suggested that the mercaptans, including methyl mercaptan (methanethiol, MeSH) and ethyl mercaptan (ethanethiol, EtSH), are highly reactive compounds with low aroma thresholds and very low boiling points. These mercaptans are observed to form during fermentation in association with hydrogen sulphide. Their suppression by DAP suggests that they are produced as by-products of yeast metabolism of methionine (Rauhut, 1993). Ethanethiol can be formed by the reaction of hydrogen sulphide with ethanol or acetaldehyde, however, this could not be confirmed up to now (Rankine, 1963, 1968 a; Amerine et al., 1980; Rauhut, 1993, 2003). The aroma of EtSH is described as “onion” or “rubber” with a threshold value of 1.1 μg L^{-1} in wine (Bartowsky & Pretorius, 2009). Fermentation studies suggest that MeSH, which gives wines rotten egg or cabbage aromas, is derived from methionine, cysteine, S-methylmethionine and sulphate (De Mora et al., 1986; Rauhut, 1993). The odour threshold of MeSH is extremely low at 0.02-2 μg L^{-1} in water (Bartowsky & Pretorius, 2009).

An accelerated high formation of H_2S leads to an increase of thioacetic acid esters, MeSAc formed in the early phase of fermentation, while EtSAc formed at the end (Rauhut, 2009). These thioacetic acid esters were probably produced through the reaction of the thiols and acetyl coenzyme A (Matsui & Amaha, 1981; Walker & Simpson, 1993). Rauhut (1996) found that the addition of MeSH and EtSH during fermentation leads to the corresponding thioacetic acid esters. Matsui & Amaha (1981) supposed that high concentration of methanethiol and H_2S can hinder the growth of yeasts. It is therefore assumed that the formation of thioacetic acid esters is a detoxification process to transform sulphur components with a free SH-group, which can inhibit enzymes, to non-affecting compounds like the thioacetic acid esters. MeSAc can be detected in normal wine in a concentration up to about 20 μg L^{-1} (Leppänen et al., 1979, 1980). Rauhut (1996) demonstrated that more than 130 μg L^{-1} could be detected in off-flavour wines. These thioacetic acid esters can hydrolyze during wine storage like other acetic acid esters after fermentation due to the chemical equilibrium (Rapp, 1998) and leads to free thiols (e.g. MeSH and EtSH) and acetic acid. A treatment of wine with copper sulphate has no effect on the concentration of the thioacetic acid esters as it mainly reacts with H_2S and thiols (Rauhut, 1996, 2003). However, the formation of these thiols and their esters in conjunction with H_2S has been observed and it was noted that their production was suppressed by DAP in nitrogen responsive strains (Rauhut et al., 1996).

The wine yeast *Saccharomyces cerevisiae* is mainly responsible for the production of several volatile sulphur compounds during the alcoholic fermentation. Therefore, it could be demonstrated that yeast strains differ in their formation of sulphur compounds (Rauhut & Kürbel, 1994; Rauhut et al., 1996, 1997, 2000 b). The origin of DMS is not clear but it might be formed by yeast from cysteine, cystine or glutathione or from dimethyl sulphoxide by a

yeast reductase (Rauhut, 1993; Ribéreau-Gayon et al., 2006 b). Some authors described DMS as “corn”, “molasses” or “asparagus” (Rauhut, 1993; Mestres et al., 2000), others as “quince” or “truffle”, when the concentration in wines is near the olfactory threshold; at higher amounts the wine gains aromas related with metallic characteristic (Anocibar Beloqui, 1998). It has been shown that DMS level increases during wine ageing and contributes to the “ageing bouquet” and it could also enhance fruity character in low concentration (De Mora et al., 1986, 1987; Rauhut, 1993). The concentration of DMS in wines ranges from 0-474 $\mu g\ L^{-1}$ or even higher, therefore it is well above the odour threshold of 25 $\mu g\ L^{-1}$ (white wine) (Goniak & Noble, 1987) and 25 $\mu g\ L^{-1}$ (red wine) (De Mora et al., 1987).

Diethyl sulphide (DES) occurs in wine and has a reminiscent of “cooked vegetables”, “onion” and “garlic” (Goniak & Noble, 1987; Swiegers et al., 2005 a; Bartowsky & Pretorius, 2009). Its flavour threshold is 0.92-0.93 $\mu g\ L^{-1}$, and its level in wines ranges from 4.1-31.8 $\mu g\ L^{-1}$ (Goniak & Noble, 1987; Swiegers et al., 2005 a). DMDS, which is characterized as cooked and intensively onion-like, was determined in white wines at concentrations from traces to 2 $\mu g\ L^{-1}$ and in red wines from 0.3-1.6 $\mu g\ L^{-1}$ (Leppänen et al., 1979, 1980; Swiegers et al., 2005 a). These small concentrations of DMDS can be formed from MeSH by oxidation. Higher amounts arising from pesticides can occur in sulphurous off-flavour wines. In addition, yeasts have the ability to reduce disulphides, such as DMDS, to mercaptans (Swiegers et al., 2005 a). Diethyl disulphide (DEDS), which was described as reminiscent of garlic and burnt rubber, can be produced by the oxidation of two molecules of EtSH. It has a threshold of 4.3 $\mu g\ L^{-1}$ in white wine and was determined in wine at concentrations from traces to 85 $\mu g\ L^{-1}$ (Goniak & Noble, 1987; Swiegers et al., 2005 a). Thiols can be also oxidized to disulphides or trisulphides, which contribute to odours like “rubber” or “garlic”. These sulphides cannot be removed by copper fining (Maujean, 2001; Swiegers et al., 2005 a).

Carbon disulphide (CS_2), which has a “sweet” and “ethereal” odour, was found from traces to 10 $\mu g\ L^{-1}$ in white wines (Leppänen et al., 1980). In the headspace of some white wines levels up to 15-18.9 $\mu g\ L^{-1}$ of CS_2 were reported. It has no significant influence on the flavour of wine as the threshold value is above the level found in wines. Carbonyl sulphide (COS), which is an odourless compound, was detected in wine from traces to 2.5 $\mu g\ L^{-1}$ (Spedding et al., 1983; Mestres et al., 2000). This compound is also unlikely to have an influence on the sensory quality of wines, because it is a colourless and odourless gas (Rauhut, 1993; Mestres et al., 2000). It is though that both compounds can result from the degradation of fungicides like ethylenebisdithiocarbamate and tetramethylthiuramdisulphide (Schmitt, 1987; Rauhut, 1993). Additionally, it was postulated that COS can be formed by the reaction of SO_2 and CO_2 (Shaw & Nagy, 1981). **Table 2-5** lists the sulphur containing compounds, including thiols, commonly found in wine (Bell & Henschke, 2005).

Table 2-5 The sulphur containing compounds, including thiols, commonly found in wine

S-compounds	Concentration in wine (μg L^{-1})	Aroma threshold (μg L^{-1})	Aroma descriptor
Carbonyl sulphide	Trace - 2.4	-	Odourless
Carbon disulphide	Trace - 18	> 37.8	rubber, chokingly repulsive, cabbage
Diethyl sulphide	4.1 - 31.8	0.93	cooked vegetables, onion, garlic
Diethyl disulphide	Trace - 85	4.3	garlic, burnt rubber
Dimethyl sulphide	Trace - 474	25	asparagus, corn, molasses low concentration: quince, truffle
Dimethyl disulphide	Trace - 85	20 - 45	cooked cabbage, intense onion
Hydrogen sulphide	Trace - 80	10 - 80	rotten egg, yeast flavour
Benzothiazole	11	50	Rubber
Ethanethiol	1.9 -18.7	1.1	onion, rubber, natural gas
2-Furanmethanethiol	0 - 350 ng L^{-1}	1 ng L^{-1}	roasted coffee, burnt rubber
Methanethiol	Trace - 16	0.02 - 0.3	cooked cabbage, onion, putrefaction
4-Methylthiazole	Trace - 11	55	green hazelnut
3-Methylthio-1-propanol	140 - 5000	500	cauliflower, cabbage, potato
4-Methyl-4-sulphanlypentan-2-one (4MSP)	Trace - 40 ng L^{-1}	0.8 - 3	cat urine, box tree, blackcurrant, broom, passion fruit
4-Methyl-4-sulphanlypentan-2-ol (4MSPOH)	18 - 22 ng L^{-1}	55 ng L^{-1}	citric, passion fruit, box tree, broom
Acetic acid 3-sulphanylhexyl -ester (3SHA)	1 - 724 ng L^{-1}	4 ng L^{-1}	passion fruit, grape fruit, citrus zest. Riesling-type note
3-Sulphanylhexanol (3SH)	50 -12822 ng L^{-1}	60 ng L^{-1}	passion fruit, grape fruit
3-Sulphanyl-2-methylpropan-1-ol	250 - 10000 ng L^{-1}	3000 ng L^{-1}	fruity, animal, sweat, broth
3-Sulphanyl-3-methylbutan-1-ol	20 - 150 ng L^{-1}	1500 ng L^{-1}	cooked leeks
Thiophene-2-thiol	0 -11	0.8	burnt rubber, roasted coffee

Source: Mestres et al. (2000), Bell & Henschke (2005), Dubourdieu et al. (2006) and Ribéreau-Gayon et al. (2006 b)

In addition, methionine can be metabolized by yeast through the Ehrlich pathway to form sulphur-containing fusel alcohol, methionol or 3-methylthio-1-propanol, which has “cauliflower” and “cabbage” odours. It was detected in wines and range from 140-5000 μg L^{-1}, it and has a threshold of 500 μg L^{-1} in wines (Rauhut, 1996; Mestres et al., 2000; Bell & Henschke, 2005). This compound can be converted further to 3-methylthiopropyl acetic acid

ester, which has a “mushroom” or “garlic” odour. It has also been proposed that 4-methylthio-1-butanol with an “onion” or “garlic” odour and 2-mercapto-1-ethanol with a “poultry” or “farmyard” odour can be biosynthesised by yeast in the same way by using the amino acids homocysteine and cysteine, respectively (Mestres et al., 2000). Some observations suggest that concentrations in wine may be influenced by the level of methionone as well as the assimilable nitrogen concentrations in the must (Rauhut, 1996; Moreira et al., 2002; Bell & Henschke, 2005).

2.5 Passion fruit (*Passiflora edulis* Sims)

Passion fruit, *Passiflora*, is the most important genus in the family *Passifloriaceae* and it is distributed within most of the tropical and sub-tropical regions of the world. Approximately 460 species of the genus *Passiflora* have already been described. From these, about 90 % have originated from America and close to 150 species occur in Brazil and Ecuador (Crochemore et al., 2003). The economical importance of some species is associated to the quality of the fruits for consumption and medicinal properties (De Neira, 2003).

In the last ten years, there has been a great increase in the international consumption of tropical fruits. According to FAO, the market for tropical fruit juices, including passion fruit, is about US$ 1 billion. This consumption reflects a growing concern with more natural and healthy nutrition mainly in Europe and the United States (Sandi et al., 2004). Hawaii, Brazil, Colombia, Ecuador, Peru, Australia, Fiji Islands, Kenya, South Africa, Papua New Guinea, New Zealand, Venezuela, India, Sri Lanka, Philippines, Taiwan, Brazil, Colombia, Ecuador and Peru were the largest producers that supply the major part of the world market, while the United States and European countries are the main importers, with a growing demand for the products (Loeillet, 1995; Somogyi et al., 1996). There are two important commercial varieties of *Passiflora edulis* Sims, e.g. purple passion fruit (*Passiflora edulis*) and yellow passion fruit or maracuja (*Passiflora edulis* Sims f. *flavicarpa* Degner). Because of its more desirable flavour and because it is sweeter, the former is preferred for consumption as fresh juice. The latter has larger fruits, higher juice yield and more acidity, therefore it is considered better suited for processing and is one of the most popular and best known tropical fruits (Martin & Nakasone, 1970; Morton, 1987; Bora & Narain, 1997; Chassagne et al., 1999; Deliza et al., 2005).

Yellow passion fruit (YPF) has round to oval shape, yellow skin at maturity and produces generous amounts of juice or pulp (Muller et al., 1964). It is known for its natural attractive colouring, unique flavour properties and medicinal purposes. It has not only high amounts of vitamin A, vitamin C, niacin, riboflavin, potassium, dietary fibre, carotenoids and polyphenolic compounds (Morton, 1987; De Neira, 2003), but also is the best tropical fruit having a floral, fruity and estery aroma with an exotic tropical sulphury note (Engel & Tressl, 1991; Werkhoff

et al., 1998; Chassagne et al., 1999; Engel, 1999; Tominaga & Dubourdieu, 2000). It contains also several non-nutritive phytochemicals that make it a tasteful and healthy addition to the diet. However, its high acidity, especially yellow passion fruit, limits its use as an ingredient in the formulation of various preparations such as beverages, ice cream, marmalade and cocktails. Passion fruit can be recommended as raw material for production of dessert and good quality fruit wine (Czyhrinciw, 1966; Fang et al., 1986; Somogyi et al., 1996; Deliza et al., 2005; Srisamatthakarn et al., 2010). In addition, it has been used widely in folk medicine in South America to treat anxiety, insomnia, asthma, bronchitis and urinary infection (Zibadi & Watson, 2004)

Passion fruit juice is a high acidic food, pH 2.8-4 and total acidity 43-51.1 g L^{-1}, which contains the predominance of two organic acids, citric acid (46.3-55 g L^{-1}) and malic acid (6.5-10.5 g L^{-1}). It also has total soluble solid 13-14.4 ^{o}Brix and contains about 145-152 g L^{-1} sugar, including glucose, fructose and sucrose (Jagtiani et al., 1988; Srisamatthakarn et al., 2010; www.thainutri.com/juicelineframe.htm, 2009). Passion fruit juice provides a good source of various nutrients (**Table 2-6**) such as vitamin C (182 mg Kg^{-1} of edible portion), vitamin A (7000-24100 IU Kg^{-1} of edible portion) and potassium (2780 mg Kg^{-1} of edible portion) (De Neira, 2003; www.hort.purdue.edu/newcrop/morton/passionfruit.html#Storage, 2009). Thirteen different carotenoids In passion fruit have been identified, e.g. phytoene, phytofluene, ζ-carotene (principal carotenoid), neurosporene, β-carotene, lycopene, prolycopene, monoepoxy-β-carotene, β-cryptoxanthin, β-citraurin, antheraxanthin, violaxanthin, and neoxanthin (Pruthi & Lal, 1958; Cecchi & Rodriguez-Amaya, 1981; Gross, 1987; Mercadante et al., 1998). Other non-nutritive phytochemicals found in passion fruit are polyphenolic compounds, which have been found to have antioxidant activity (Salah et al., 1995; Rice-Evans & Miller, 1996) as well as anticancer properties (Yoshida et al., 1992; Kang & Liang, 1997), however, the flavonoids have not been reported (Talcott et al., 2003). The total carotenoid concentration of passion fruit is 932 mg L^{-1}, of which only three individual carotenoids, α-carotene, β-carotene as well as β-cryptoxanthin (350, 5250 and 460 g Kg^{-1} of edible portion, respectively) had been quantified (De Neira, 2003). Other phytochemicals conclusively identified include aroma compounds such as volatile thiols, terpenes, fatty acid esters, alcohols, and various other aromatics (Engel and Tressl, 1991; Werkhoff et al., 1998; Chassagne et al., 1999; Engel, 1999; Tominaga & Dubourdieu, 2000).

Table 2-6 The chemical compositions of the yellow passion fruit (per 100 g of edible portion)

Chemical compounds	Concentration range (mg)
Protein	670
Total fat	180
Carbohydrate	14450
Sugar (g L^{-1})	145 - 152
Total fibre	0.02
Calcium	4
Iron	0.36
Magnesium	17
Phosphorus	25
Potassium	278
Sodium	6
Zinc	0.06
Copper	0.05
Selenium	0.10
Vitamin C	18.20
Thiamine	n.d.
Riboflavin	0.10
Niacin	2.24
Food - Folate (μg)	8
Vitamin B6	0.06
Vitamin B12 (μg)	n.d.
Vitamin A (IU)	700 - 2410
Total carotenoids (mg L^{-1})	932
α-Carotene (g)	35
β-Carotene (g)	525
Vitamin E	n.d.
Vitamin K (μg)	n.d.
Saturated fat	10
Monounsaturated fat	20
Polyunsaturated fat	110
Cholesterol	n.d.
Citric acid (g L^{-1})	46.3 - 55.0
Malic acid (g L^{-1})	6.5 - 10.5

n.d.: not detectable

Source: Jagtiani et al. (1988), De Neira (2003)

www.nutritionanalyser.com/food_composition/?fid=09233

Passion fruit is loaded with amino acids such as arginine, aspartic acid, glycine, leucine, lysine, proline and threonine. Tyrosine and valine are all found in varying amounts in the different varieties of the passion fruit (www.acai-natural-pain-free-health.com/passion-constituents.html, 2010). It has been determined that passion fruit contains various free amino acids (both L-amino acid and D-amino acid) as shown in **Table 2-7** and it has been reported that yellow passion fruit contained high concentrations of glutamine, glutamic acid, aspartic acid, arginine, aspartic acid, glycine, serine and alanine (Brückner & Westhauser, 2003).

Table 2-7 Quantities of amino acid (AA) enantiomers in yellow passion fruit

Amino acids	L-amino acid (μmol L^{-1})	D-amino acid (μmol L^{-1})
Alanine	975	12.0
Arginine	752	5.9
Asparagine	195	n.d.
Aspartic acid	3,065	16.2
Glutamine	4,336	n.d.
Glutamic acid	3,558	17.1
Glycine	1,317	n.d.
Histidine	480	n.d.
Isoleucine	346	n.d.
Leucine	186	n.d.
Lysine	356	n.d.
Methionine	113	n.d.
Phenylalanine	344	n.d.
Serine	2,962	17.1
Threonine	297	n.d.
Tryptophan	92	n.d.
Tyrosine	142	n.d.
Valine	456	n.d.

n.d.: not detectable

Source: Brückner & Westhauser (2003)

The powerful volatile constituents of passion fruit have been investigated by several research groups over the past decades. They have resulted in the characterization of a broad spectrum of volatile constituents in comprehensive reviews (Pruthi & Lal, 1959; Winter & Klöti, 1972; Casimir et al., 1981; Whitfield & Last, 1986; Shibamoto & Tang, 1990; Werkhoff et al., 1998). To date, more than 200 components have been identified as components of the

flavour in passion fruit like esters; terpenes and norisoprenoids, which are present in both free and glycosylated form; glycosides of benzyl alcohol; 3-methyl-2-buten-1-ol, and mandelonitrile (Parliament, 1972; Casimir et al., 1981; Chen et al., 1982; Whitfield & Last, 1986; Shibamoto & Tang, 1990; Chassagne et al., 1996 a, 1996 b, 1999; Winterhalter, 1990; Engel & Tressl, 1983; Brat et al., 2000). Jordán et al. (2000) investigated the aromatic profile of yellow passion fruit aqueous essence. Recently, Jordán et al. (2002) reported that the most abundant compounds in yellow passion fruit juice and aqueous essence are linalool, fatty acid ethyl ester (butanoic, octanol and hexanoic acid ethyl ester). These authors reported a total of 62 compounds and some most abundant compounds were identified like linalool, octanol, butanoic and hexanoic acid ethyl ester, 3-methyl-2-butanone; lactic acid ethyl ester, malonic acid diethyl ester, 3-penten-2-ol, 1,3-dimethyl benzene, and hexanoic acid 2-methylbutyl ester. More recently, the enantiomeric compositions of the acetic, butanoic, hexanoic and octanoic acid esters of the secondary alcohols 2-pentanol, 2-heptanol, and 2-nonanol were identified in yellow passion fruits (*Passiflora edulis* f. *flavicarpa*) and the preparation of these ester via lipase-catalyzed esterification was also investigated (Strohalm et al., 2007, 2010).

Sulphur containing compounds including volatile thiols also play important roles in the flavour of yellow passion fruit. These sulphur-containing components possess high odour intensities and low threshold values. To date, the presence of 47 sulphur-containing components in this fruit has been reported (Werkhoff et al., 1998; Engel, 1999). The 3-(methylthio)hexanol and a mixture of *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane (MPO) were the first representatives described as key odorants in the aroma of the yellow passion fruit (Winter et al., 1976). Several approaches have been described to synthesize the optical isomers of 3-(methylthio)hexanol and MPO and to describe their sensory properties in this fruit (Heusinger & Mosandl, 1984; Mosandl & Heusinger, 1985; Singer et al., 1986) and it could be demonstrated that odour quality of YPF is strongly influenced by chirality. Heusinger & Mosandl (1984) investigated that only (S)-3-(methylthio)hexanol has an exotic, fruity odour, while the (R)-enantiomer is described as herbaceous and weak. Later, Engel & Tressl (1991) described for the first time the presence of 3SH and the esters of acetic, butanoic, and hexanoic acid of both 3SH and 3-(methylthio) hexanol. Additionally, these compounds have also been expected to be key aromas of the yellow variety.

In 1994, significant differences were revealed for the enantiomers of the sulphur-containing esters (acetic, butanoic and hexanoic acid esters) of 3SH and 3-(methylthio)hexanol, only the (R)-configurated esters of 3SH exhibit the tropical fruit notes of yellow passion fruit, whereas for 3-(methylthio)hexanol the (S)-esters are more intensive with pronounced sulphury character (Weber et al., 1994, 1995). In addition, acetic acid 3-sulphanylhexyl ester (3SHA) and butanoic acid 3-sulphanylhexyl ester (3SBA) were shown to be present in yellow passion

fruits in almost optically pure form (> 95 % (S)-ester) (Weber et al., 1995). Recently, the other 35 sulphur-containing flavour components have been identified in yellow passion fruits for the first time, such as acetic acid 3-(methylthio)propyl (acetic acid methionyl ester, which contributes to herbaceous odour impressions and a typical vegetable-like character), butanoic and hexanoic acid methionyl ester (odour threshold 10−20 and 500 μg L^{-1} in water, respectively), methyl and ethyl ester of 3-(methylthio)propionic acid (odour threshold 150 and 300 μg L^{-1} in water, respectively) and the whole series of propyl, butyl, isobutyl, *sec*-butyl, pentyl, 2-methylbutyl, 3-methylbutyl, hexyl, and (*Z*)-3-hexenyl esters of 3-(methylthio)-propionic acid (Werkhoff et al., 1998). In general, the 3-(methylthio)propionic acid esters have a sulphury, vegetable-like odour, and only hexyl 3-(methylthio)propionic ester with its fruity and geranium-like odour note may contribute to the overall olfactory impression. In addition, threshold values of 3-(methylthio) esters of propanoic acid in water are significantly higher compared with other sulphur compounds. For example, butyl 3-(methylthio)propionic acid ester has a taste threshold in water of 1−2 mg L^{-1}. In 2000, 3MSB and acetic acid ester of 3MSB (3SMBA) as well as the precursor of 3SH as trimethylsilylated *S*-3-(hexan-1-ol)-L-cysteine have been identified in passion fruit for the first time (Tominaga & Dubourdieu, 2000). This research also suggested that 3SH in passion fruit juices is present in both free and conjugate form, additionally both 3SH and 3MSB may be produced in vitro from nonvolatile extracts of this fruit by the enzymatic action of a cell-free extract of *Eubacterium limosum*, which has a β-lyase activity on *S*-cysteine conjugates. More recently, a new simple route to (R)-3-sulphanylhexan-1-ol and its immediate derivative (1)-*cis*-2-methyl-4-propyl-1,3-oxathiane, which are the main component procedure responsible of the passion fruit aroma, has been investigated (Scafato et al., 2009).

It has been reported that a dry table wine prepared from yellow passion fruit contained various volatile substances such as isobutyl alcohol, isoamyl alcohol, active amyl alcohol, acetaldehyde, acetic acid ethyl ester and hexanoic acid ethyl ester, etc. (Muller et al., 1964). The use of different *Saccharomyces* yeast strains for passion fruit wine fermentation has been shown to result in wines with different fermentation kinetics and the formation of secondary metabolites, such as SO_2-binding compounds, some organic acids, acetic acid esters, fatty acid ethyl esters and higher alcohols (Srisamatthakarn et al., 2010) However, there is little research work on passion fruit wine production as well as improvement of its quality, especially the compounds responsible for the typical passion fruit wine aroma still need to be investigated. In addition, only little information is available on the improvement of passion fruit wine quality by optimal choice of yeasts as well as nutrient supplementation, additionally, nearly no information is available to optimize the release and preservation of volatile flavours in passion fruit wine during alcoholic fermentation.

3. MATERIALS AND METHODS

Raw materials, yeast strains, yeast nutrients, media, chemical reagents, enzymes, devices and equipments applied in this study are listed in this section. Yeast cultivation and enumeration, analysis methods, and experimental designs are described in the following text.

3.1 Raw materials

3.1.1 Grape musts

White grape of Scheurebe or Seeding 88 (*Vitis vinifera* L. cultivar Scheurebe) and Sauvignon blanc (*Vitis vinifera* L. cultivar Sauvignon blanc) were used in this study. It was long assumed that Scheurebe was *V. vinifera* cultivar Silvaner x *V. vinifera* cultivar Riesling, but DNA analysis in the late 1990s ruled out Silvaner as a parent, while confirming Riesling as the father. It is widely grown in the Palatinate, Rhein-Hesse and Nahe regions of Germany (www.vivc.de/datasheet/dataResult.php?data=10818, 2010).

Fresh Scheurebe and Sauvignon blanc grape juices were obtained from the Department of Grapevine Breeding and Grafting, Geisenheim Research Center, Geisenheim, Germany from 2007-2009 harvest seasons.

3.1.2 Passion fruit (*Passiflora edulis* Sims) puree

The frozen YPF puree employed for this study is the hybrid breeding variety of *Passiflora edulis* Sims *f. flavicarpa* Degner. It was obtained from Thai Nutri-Juice Co., Ltd., Thailand and kept at -18 ^{o}C until use.

3.2 Yeast strains employed for wine making in industry

Eighteen commercial *Saccharomyces* yeast strains were used in this study (**Table 3-1**). They were obtained from Lallemand, Danstar Ferment AG, Zug, Switzerland; DSM Food Specialities, Delft, The Netherlands; Laffort Oenologie, Bordeaux, France; Erbslöh Geisenheim AG and La Littorale - Groupe Erbslöh, Geisenheim, Germany; Anchor-Bio Technologies, Eppindust, South Africa and AB Mauri, Mauri Yeast Australia, Toowoomba, Australia. The enological properties of the commercial yeast strains used in the study are shown in **Table 3-2**.

Table 3-1 List of the commercial yeast strains used in the study

Strains	Code*
Saccharomyces cerevisiae (var. *bayanus*), Lallemand – Lalvin EC-1118 (Prise de Mousse)™	EC1118
Saccharomyces cerevisiae, Lallemand-Enoferm Simi White™	Simi White
Saccharomyces cerevisiae (var. *bayanus*), Lallemand-Enoferm QA23™ (YSEO® process)	QA23
Saccharomyces cerevisiae, Lallemand–Uvaferm SVG™	SVG
Saccharomyces cerevisiae (hybrid), Lallemand–Cross Evolution	Cross Evolution
Saccharomyces cerevisiae, DSM-Collection Cépage Sauvignon®	Sauvignon
Saccharomyces cerevisiae, DSM–Fermicru® 4F9	4F9
Saccharomyces cerevisiae, DSM–Fermicru® LVCB	LVCB
Saccharomyces cerevisiae, Laffort–Zymaflore VL3	VL3
Saccharomyces cerevisiae (breeding), Laffort–Zymaflore X5	X5
Saccharomyces cerevisiae (breeding), Laffort–Zymaflore X16	X16
Saccharomyces cerevisiae (hybrid), Anchor-VIN7	VIN7
Saccharomyces cerevisiae (hybrid), Anchor-VIN13	VIN13
Saccharomyces spp. (blend), Anchor–Alchemy I	Alchemy I
Saccharomyces spp. (blend), Anchor–Alchemy II	Alchemy II
Saccharomyces cerevisiae (var. *bayanus*), Maurivin™ AWRI R2	AWRI R2
Saccharomyces cerevisiae (var. *bayanus*), La Littorale-LittoLevure Sauvignon	LittoLevure
Saccharomyces cerevisiae (var. *bayanus*), Erbslöh-Oenoferm® Freddo	Freddo

* Character abbreviation used throughout the text.

Table 3-2 Enological properties of eighteen commercial yeast strains used in the study

Yeasts	Temperature (^{o}C)	Nitrogen requirement	Ethanol tolerance (% v/v)	Volatile acidity (g L^{-1})	Glycerol (g L^{-1})	H_2S (μg L^{-1})	SO_2 (mg L^{-1})	References
EC1118	15 – 25	low	18	low to average	-	low	average	www.lalvinyeast.com/images/library/EC1118_Yeast.pdf
Simi White	15 – 30		15	-	-	low		www.lallemandwine.us/products/yeast_chart.php
QA23	15 – 32	low	16	< 0.2	low	very low	low	www.lallemandwine.com/catalog/img/catalog/description_activity_image_1209025085_QA23 eng.pdf
SVG	16 – 25	medium	15	low		low	low	www.lallemandwine.com/catalog/products/view/61
Cross Evolution	10 – 20	low	16	-	-	-	-	www.lallemandwine.us/products/yeast_chart.php
Sauvignon	12 – 25	-	15	< 0.2	6 - 8	-	medium	www.dsm.com/en_US/downloads/oenology/CCSauvignon.pdf
4F9	15 – 25	-	15.5	< 0.15	5 - 7	low	none	www.dsm.com/en_US/downloads/oenology/Fermicru_4F9.pdf
LVCB	12 – 20	-	15	< 0.2	5 - 7	low	none	www.dsm.com/en_US/downloads/oenology/FermicruLVCB.pdf
VL3	15 – 21	high	14.5	low	-	low	-	www.laffort.com/en/quality-management/74
X5	13 – 20	medium	16	low	-	low	-	www.laffort.com/en/products/zymaflore-yeasts/70
X16	12 – 20	low	16	low	-	low	-	www.laffort.com/en/products/zymaflore-yeasts/71
VIN7	12 – 20	low	15	0.4 – 0.8	5 -7	-	very low	www.anchorwineyeast.com/envin7.html
VIN13	15 – 20	low	16.5	< 0.3	5 -7	-	very low	www.anchorwineyeast.com/envin13.html
Alchemy I	13 – 20	low to average	15.5	< 0.5	5 -7	-	very low	www.anchorwineyeast.com/enalch1.html
Alchemy II	13 – 20	average	15.5	< 0.5	5 -7	-	very low	www.anchorwineyeast.com/enalch2.html
AWRI R2	11 – 25	medium	15	< 0.3		-		www.maurivinyeast.com/media/35.pdf
LittoLevure	13 – 21	-	15	< 0.25	5 - 8	-	low	www.lalittorale.com/en/oehefe_en.php
Freddo	13 – 17	low	15	-	-	-	low	www.erbsloeh.com/en/datenblatt/Saft/Oenoferm_Freddo.pdf

Yeast culture rehydration and preparation

Active dried yeasts were rehydrated in tap water (1 g per 5-10 ml water) at approximately 35-40 ^{o}C for 10-30 min prior to inoculation at 0.02-0.30 % to the juice following the recommendations of the manufacturer indicated on the package.

3.3 Yeast nutrients

Eight commercial yeast nutrients were used in this study (**Table 3-3**). They were obtained from Lallemand, Danstar Ferment AG, Zug, Switzerland; Laffort Oenologie, Bordeaux, France and Erbslöh Geisenheim AG, Geisenheim, Germany.

Table 3-3 List of the commercial yeast nutrients used in the study

Yeast nutrient	Code*
Natural inactivated yeast with high glutathione, Lallemand-OptiWhite®	OptiWhite
Blend of inactivated yeast fractions, Lallemand-Fermaid® O	Fermaid O
Blend of diammonium hydrogen phosphate, thiamine, yeast cell walls and ammonium sulphate, Lallemand-Fermaid® E blanc	Fermaid E
Blend of inactivated yeast and yeast cell walls, Laffort-Superstart®	Superstart
Diammonium hydrogen phosphate [$(NH_4)_2HPO_4$], Erbslöh-Vitamon® A	DAP
Thiamine, Erbslöh-Vitamon® B	Thiamine
Blend of diammonium hydrogen phosphate and thiamine, Erbslöh-Vitamon® Combi	VCombi
Blend of diammonium hydrogen phosphate, thiamine, yeast cell walls and yeast-stimulating cell parts, Erbslöh-Vitamon® Ultra	VUltra

* Character abbreviation used throughout the text.

Nutrient preparation

Each nutrient was dissolved in water (10-20 times) according to the concentration of each experimental plan prior to its addition into juice. Exceptionally, Superstart was dissolved in tap water (1 g per 15 ml water) at approximately 35-40 ^{o}C following the recommendations of the manufacturer indicated on the package prior to its addition into a rehydrated yeast suspension prepared before.

3.4 Chemical reagents and enzymes

Chemical reagents

Ammonium sulphate, ammonium, carbon disulphide (CS_2), copper sulphate pentahydrate, dihydrogen phosphate, disodium hydrogen phosphate dodecahydrate, ethylene diamine tetraacetic acid disodium salt dihydrate (EDTA-$Na_2H_2.2H_2O$), dichoromethane, glucose, glycerine, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), hydrochloric acid, L-cysteine hydrochloride anhydrous, magnesium sulphate heptahydrate, methylene blue, potassium dihydrogen phosphate, potassium metabisulphite, sodium hydrogen carbonate, sodium potassium tartrate, sodium thiosulphate, starch soluble, sulphuric acid and triethanolamine hydrochloride were obtained from Merck KGaA, Darmstadt, Germany. Citric acid monohydrate, dithiothreitol, glycine, glutamate dehydrogenase, dimethyl dicarbonate (DMDC), 2-oxoglutaric acid disodium salt, phosphooenopyruvic acid monosodium salt monohydrate and sodium ρ-hydroxymercuribenzoate (ρ-HMB) were from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany. Acetic acid ethyl ester, acetic acid 2-phenyl ethyl ester, benzene acetic acid ethyl ester, butyric acid ethyl ester, cumene, decanoic acid, ethanethiol (EtSH), decanoic acid ethyl ester, hexanoic acid hexyl ester, 1-hexanol, lactic acid ethyl ester, linalool, 2-methyl butanol, 3-methyl butanol, octanoic acid, 2-pheyl ethanol, propionic acid ethyl ester, sodium hydroxide, sodium sulphate monohydrate, succinic acid diethyl ester, α-terpineol, MeSAc, tran/cis-linalool oxide and BHA (3-*tert*-butyl-4-hydroxyanisole) were from Fluka Chemie GmbH, Buchs, Switzerland.

2,6-Dimethyl-5-hepten-2-ol (DMH), D-fructose, ethyl alcohol (p.a.), methyl alcohol, sodium acetate and Tris-(hydroxymethyl) aminomethane were obtained from Carl Roth GmbH, Karlsruhe, Germany. Glycylglycine was from AppliChem GmbH, Darmstadt, Germany. Potassium iodide and i-butanol were obtained from Riedel-de Haën, Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany. Iso-butyric acid ethyl ester, acetic acid 3-methyl butyl ester, acetic acid 2-methyl butyl ester, DEDS, ethyl methyl sulphide (EMS), hexanoic acid ethyl ester, methanethiol sodium salt (MeSH), octanoic acid ethyl ester and thioacetic acid S-propyl ester (PrSAc) were obtained from Aldrich Chemical Company Ltd., Gillingham, England. DMDS, DMS, DMTS, and 3SH were obtained from Acros Organics, Geel, Belgium. EtSAc was from Alfa Aesar GmbH & Co KG, Karlsruhe, Germany. Methyl isopropyl sulphide was obtained from Alfa/Chemie Inc., Delaware, U.S.A. Butylmethyl sulphide (MBS), and methyl-isopropyl sulphide (i-MPS) were obtained from Lancaster, Walkerburn, England. 3SHA, 4-methoxy-2-methyl-2-sulphanylbutane (4,2,2MSB) and 4MSP were obtained from CHEMOS GmbH, Regenstauf, Germany.

Enzymes and media

Arginase, lactate dehydrogenase and pyruvate kinase were obtained from Fluka, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany. Adenosine tripohosphate, glutamate dehydrogenase, glycerokinase, L-lactate dehydrogenase, nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate disodium salt were obtained from Roche Diagnostics GmbH, Penzberg, Germany. Aldehyde dehydrogenase was obtained from VWR International GmbH, Darmstadt, Germany. Urease was obtained from Serva Electrophoresis GmbH, Heidelberg, Germany.

3.5 Fermentation kinetics

Fermentation kinetic was obtained by monitoring carbon dioxide production during yeast growth. The amount of carbon dioxide released was determined by weight loss every 1-2 days.

3.6 Analytical methods

The analytical analyses which were used in the study are described as follows.

3.6.1 Physico-chemical analytical methods

pH: The pH was analysed by pH meter (pH 526 Multical®, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Physico-chemical analytical method with FTIR spectrophotometry: Ethanol, reducing sugar, glycerol, tartaric acid, malic acid, lactic acid, total acidity, volatile acidity and pH were analyzed by FTIR spectrometry as described in Baumgartner et al. (2001) and Patz et al. (1999). They were performed at the Department of Wine Analysis and Beverage Research, Geisenheim Research Center, Germany.

Sugar: Residual sugar, reducing sugar and inverted sugar (as glucose) were analysed by the Rebelein method according to Iland et al. (1993).

Sulphur dioxide: Free and total sulphur dioxide in wine were determined by the Ripper titrametric method according to Zoecklein et al. (1999) and by FIAstar^{TM} 5000 following the instructions of the manufacturer. These analysis were performed at the Department of Wine Analysis and Beverage Research, Geisenheim Research Center, Germany.

Amino nitrogen: A modified procedure from Wylie and Johnson (1961) was used to quantify free alpha amino nitrogen (FAN, as mg L^{-1} glycine), which is assimilable nitrogen. NOPA is an *o*-phthaldialdehyde/N-acetly-L-cysteine spectrometric assay described by Dukes & Butzke (1998) and used to detect free α-amino nitrogen (as mg L^{-1} isoleucine).

Amino acids: They were determined by the Amino Acid Analyzer S433 (Sykam GmbH, Eresing, Germany) following the instructions of the manufacturer and were performed at the Department of Soil Science, Geisenheim Research Center, Germany.

Metabolic products and amino nitrogen by enzymatic method: Acetaldehyde, glycerol, pyruvate, α-ketoglutarate and Ferm-N value (yeast-utilisable/assimilable nitrogen derived from amino acids) were determined enzymatically by an UV/VS spectrometer Lambda 2 (Perkin Elmer GmbH, Überlingen, Germany) and wavelength at 340 nm equipped with a refrigerated/heating circulator, Model F25-ME (JULABO Labortechnik GmbH, Seelbach, Germany) and controlled at 25 ^{o}C isothermic condition according to instructions of Boehringer Mannheim GmbH, Germany (1998).

3.6.2 Organic acids using a high performance liquid chromatographic method

Samples were centrifuged for 3 min at 1300 rpm and then a 5 µL sample was injected into the high performance liquid chromatography (HPLC). In some cases, it was necessary to dilute the samples 1:2 or 1:5 with ultrapure water (MilliQ, Millipore). Organic acids were determined by Hewlett Packard (HP) Series 1100 HPLC (Agilent Technologies) equipped with a multiwavelength detector (UV/Visible) according to a modified procedure from Schneider et al. (1987). Organic acids were resolved on an Allure® Organic Acids column (250 mm x 4.6 mm i.d., 5 µm particle sizes) with a security guard column cartridge C18 (4 x 3.0 mm, Fa. Phenomenex). The column was operated at a column temperature of 46 °C with diluted sulphuric acid (0.0139 % in ultrapure water) or 0.5 % ethanol (v/v) as eluent at the flow rate of 0.6 ml min^{-1}. Eluting compounds were detected by UV absorbance at 210 nm.

3.6.3 Gas chromatographic methods

3.6.3.1 Analysis of esters, higher alcohols, fatty acids and monoterpenes

Samples were extracted according to the 'Kaltron' method by liquid-liquid extraction with 1,1,2-trifluorotrichloroethane according to a modified procedure from Rapp et al. (1994). The 10 ml samples were extracted by shaking for 20 min with an addition of 2 g NaCl for better extraction, 1188 μg L^{-1} 2,6-dimethyl-5-hepten-2-ol as quantification internal standard, 112 μg L^{-1} isopropylbenzene as internal standard control and 100 μl 1,1,2-trifluorotrichloroethan (Freon 113 or Kaltron) as extracting solution. The mixture was then centrifuged for 8 min at 3000 rpm, dried over anhydrous sodium sulphate (50 mg on glass wool) and the supernatant extract was injected into the gas chromatography/mass spectrophotometry (GC-MS).

Aromatic compounds (esters, higher alcohols, fatty acids and terpenes) were analysed by gas chromatograph (Hewlett-Packard, HP 5890 Series II) equipped with a cooled injection system CIS-3 (Gerstel GmbH, Mülheim an der Ruhr, Germany) and detected by HP 5972 mass selective detector (MSD) operating in electron impact mode. The chromatographic parameters for the analysis of aroma compounds by GC-MS are summarized in **Table 3-4**.

3.6.3.2 Low boiling point of volatile sulphur containing compounds

The wine samples are stored at 4 ^{o}C before sample preparation. The GC-vials were flushed with argon gas and then cold wine samples were pipetted into the GC-vials containing 1.7 g NaCl. Regular 10 ml GC-vials filled with 5 ml sample were used as headspace sampling vials. Then, 5 μl 2,6-di-tert-butyl-4-methyl-phenol (4 mg L^{-1}) as antioxidant, 20 μl ethylenediamine tetraacetic acid (0.2 g L^{-1}) and 10 μl propanal (500 mg L^{-1}) as SO_2-binding compounds and 10 μl internal standard (6 μg S L^{-1} i-MPS and 6 μg S L^{-1} BMS) were quickly added into the samples and then analysed by gas chromatography-pulse flame photometric detector (GC-PFPD).

Low volatile sulphur compounds were analysed by an HP 6890 gas chromatograph equipped with automatic headspace sampling (Multipurpose Sampler MPS 2) and a cooled injection system CIS-4 (Gerstel GmbH, Mülheim an der Ruhr, Germany) then detected by an OI 5380 pulse flame photometric detector (PFPD) (OI Analytical, USA) according to Rauhut et al. (2005 a, 2005 b). The chromatographic parameters for the analysis of low boiling point volatile sulphur compounds by GC-PFPD are summarized in **Table 3-5**.

Table 3-4 Chromatographic parameters for the analysis of esters, fatty acids and monoterpenes in juice and wine by GC-MS as described in **3.6.3.1**

Parameter	Specification
Gas Chromatography	HP 5890 Series II equipped with a cooled injection system CIS-3
Injection	2 µl Splitless time = 1 min Temperature program: 30 °C, rate 12 °C s^{-1} 230 °C for 4 min
Column	Varian VF-5MS (60 m x 0.32 m i.d., 1 µm film thickness)
Carrier gas	Helium (1 ml min^{-1})
Temperature program	40 °C, 5 min 125 °C, rate 3 °C min^{-1} 200 °C, rate 6 °C min^{-1}, for 14.2 min
Transfer line	280 °C
Detector	HP 5972 Mass Selective Detector (MSD) SCAN mode
Source temperature	180 °C
EM-Voltage	2212
Mass range (m/z)	35 – 250 (3.43 Scans s^{-1})

The aroma compounds were identified by the retention time and comparison of the MS signal with the standard substances.

Table 3-5 Chromatographic parameters for the analysis of low boiling point sulphur containing compounds in wine samples by GC-PFPD as described in **3.6.3.2**

Parameter	Specification
Gas Chromatography	HP 6890 gas chromatograph equipped with headspace sampler (Multipurpose Sampler MPS 2) and a cooled injection system CIS-4 (Inlet liner: w/wool deactivated)
Cooled injection system	Temperature program: -100 ^{o}C, rate 12 ^{o}C s^{-1} 40 ^{o}C, rate 12 ^{o}C s^{-1} 180 ^{o}C for 8 min
Injection	1000 μl Split-ratio = 10:1 Preheating 60 ^{o}C for 45 min Syringe 63 ^{o}C
Column	SPB-1 Sulphur (Supelco 24158, Bellefonte, PEN, USA) (30 m x 0.32 m i.d., 1 μm film thickness)
Carrier gas	Helium (Constant flow rate 1.1 ml min^{-1}) Average velocity: 20 cm s^{-1} at 60 ^{o}C
Temperature program	29 ^{o}C for 7 min 180 ^{o}C, rate 10 ^{o}C min^{-1} 180 ^{o}C for 10.5 min
Detector	HP 5380 pulse flame photometric detector (PFPD)
Detector temperature	250 ^{o}C
Gas requirements	Air: 420 kPa Hydrogen 420 kPa

3.6.3.3 Volatile thiols involved in varietal aromas

The volatile thiols were specifically extracted by the reversible combination of the thiols with *p*-hydroxymercuribenzoate (*p*-HMB) and analyzed with gas chromatography coupled with mass spectrometry (GC/MS) according to a modified Ferreira et al. (2007) method.

50 ml of wine containing 200 ng L^{-1} internal standard (4,2,2MSB) and 1.25 g L^{-1} BHA were percolated through a LiChrolut EN 500mg SPE cartridge (Merck, Darmstadt, Germany) at a maximum flow of 5 ml min^{-1}. The sorbent was previously conditioned with 10 ml of dichloromethane, 10 ml of methanol and 10 ml of 13 % ethanol (v/v). The cartridge was then rinsed with 50 ml of an aqueous solution containing 40 % of methanol (v/v) buffered with TRIS 0.2 M at pH 7.2 and after this, with 5 ml of ultrapure water (MilliQ, Millipore). The sorbent was dried by forcing a stream of nitrogen (ca. 50 ml min^{-1}) to pass through the bed for 20 min. Thiols were eluted with a 10 ml solution containing 99 % dichloromethane and 1 %

methanol (v/v). The samples were purified with liquid-liquid extraction using 3 × 1 ml of an aqueous solution of *p*-HMB (1 mM in a solution of HEPES 0.2 M at pH 10.7). The 3 aliquots were combined and the pH of this aqueous phase was brought to 7.5 by the addition of 60 µl of HCl 4.6 M. Complexes were then broken with the addition of 450 µl of dithioerythritol 10 mM in HEPES and free thiols were further extracted twice, with 750 µl of dichloromethane each time. The organic phase was collected and dried on anhydrous sodium sulphate and gently concentrated by evaporation in a water bath (47 °C) to a final volume of 25 µl.The concentrated sample was transferred into a vial and it was analysed within 24 hours.

The analytical system comprised of a 6890N GC oven (Agilent Technologies, Böblingen, Germany) equipped with a cold Injection system 4 (CIS4), a Multi Purpose Sampler 2 (MPS2) both from Gerstel (Mülheim an der Ruhr) and a DB-WAX column 30 m x 0.32 mm x 0.25 µm (Agilent Technologies, Böblingen, Germany). The detector used was a 5975C mass selective detector (MSD) from Agilent Technologies (Böblingen, Germany). The Chromatographic conditions are described in **Table 3-6**.

3SH, 3SHA, and 4MSP were detected in selected ion monitoring (SIM) mode and the quantifier ions were *m*/*z* = 134, 116, and 132, respectively, whereas the internal standard was detected with the ion *m*/*z* = 100 (Ferreira et al., 2007).

Calibration: Riesling wine was dearomatized according to a modified procedure described from Fedrizzi et al. (2007). The following modifications were conducted: wine was treated two times with 3 g L^{-1} activated charcoal to remove any sulphur compounds and other main volatile compounds and then filtrated. The potassium metabisulphite (KMS) was added corresponding to 100 mg L^{-1} free SO_2. Calibration for the thiol analysis was done by adding increasing quantities of the three volatile thiol reference compounds (3SH, 3SHA and 4MSP) and internal standard (4,2,2MSB) to the dearomatized wine. For each concentration, the volatile thiols were extracted from the wine according to a modified Ferreira et al. (2007) method. The retention time windows of selected ion chromatograms of the four thiols obtained in the analysis of the dearomatized wine sample spiked with different levels of the analytes are demonstrated in **Figure A-1**. Calibration range, limit of quantification and standard deviation for each of the thiols are given below:

	3SH	**3SHA**	**4MSP**
Calibration range (ng L^{-1})	0-5000	0-500	0-100
Limit of quantification (ng L^{-1})	300	50	10
% Standard deviation	17.5 %	33.0 %	12.2 %

Table 3-6 Chromatographic parameters for the analysis of aroma compounds in wine samples by GC-MSD as described in **3.6.3.3**

Parameter	Specification
Gas Chromatography	HP 6890 gas chromatograph (Agilent Technologies, Böblingen, Germany)
Injection	3 μl, splitless for 5 min in a CIS4 (Gerstel, Mülheim an der Ruhr)
CIS4 program	Initial temperature: 40 °C 250 °C, rate 12 °C min^{-1}, hold 5 min
Column	DB-WAX capillary column (J&W, Agilent Technologies) (30 m x 0.32 mm i.d., 0.25 μm film thickness)
Carrier gas	Helium (flow rate 1.4 ml min^{-1})
Oven temperature program	60 °C for 5 min 240 °C, rate 3 °C min^{-1}, hold 20 min
Detector	HP 5975C mass selective detector (MSD) SIM Mode
Detector temperature	Source 230 ^{o}C/ 150 ^{o}C

3.7 Fermentation trials of Scheurebe grape juice

The fermentation trials of Scheurebe grape juice were performed in two sequential trials as follows.

3.7.1 Fermentation of fresh Scheurebe grape juice with two commercial *Saccharomyces* yeast strains and four commercial nutrient sources

In 2008, fermentations were carried out in fresh Scheurebe grape juice. The properties of initial grape juice were reducing sugar content 176 g L^{-1}, pH 3.2, ammonium 0.09 g L^{-1}, NOPA 193 mg L^{-1} (as isoleucine), free alpha amino nitrogen 51.9 mg L^{-1}, malic acid 6.1 g L^{-1}, tartaric acid 4.4 g L^{-1}, shikimic acid 46 mg L^{-1}, acetic acid 0.12 g L^{-1}, citric acid 0.2 g L^{-1} and total amino acids (without proline) 1314.6 mg L^{-1} (**Table A-1**). Two days before preparation of grape must, 30 mg L^{-1} of sulphur dioxide were added into the grape juice as KMS. Duplicate

experimental fermentations were carried out in 2.5-liter bottles containing 2.2 L grape juice. Two commercial *Saccharomyces* yeast strains and four different nutrient sources as well as without nutrient addition (control) were used in this study, thus giving ten different treatment combinations (**Table 3-7)**. Nutrients were added into the grape juice according to an experimental design. The yeast cultures were rehydrated following the method described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The progress of fermentation was followed by monitoring CO_2 production, which was determined by weight loss during fermentation. After the weight losses of the samples were constant, wines were cold stabilized at below 10 ^{o}C for 7 days and racked into previously cleaned bottles. Then potassium metabisulphite (KMS) was added corresponding to 80 mg L^{-1} free SO_2 in finished wine and bottled wines were stored at below 15 ^{o}C until need. After this, 150 ml wine samples, without sulphur dioxide addition, were kept at -18 ^{o}C for analysis of SO_2-binding compounds, glycerol and residual sugar. The experimental plan is demonstrated in **Table 3-7**. The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-7 Experimental design of yeast inoculation and nutrient supplementation

Saccharomyces yeast strain	Nutrient source *
EC1118	Control (without nutrient)
	OptiWhite
	Superstart
	DAP
	Fermaid E
X5	Control (without nutrient)
	OptiWhite
	Superstart
	DAP
	Fermaid E

* All the nutrients were added at the concentration of 0.3 g L^{-1}, except control treatment

3.7.2 Impact of thirteen commercial *Saccharomyces* yeast strains on fermentation characteristics and quality of Scheurebe grape wine

In 2008, fermentation was carried out in fresh Scheurebe grape juice. The composition of initial grape juice was reducing sugar content 175 g L^{-1}, pH 3.18, ammonium 0.09 gL^{-1}, NOPA 194 mg L^{-1} (as isoleucine), free alpha amino nitrogen 84.8 mg L^{-1}, malic acid 6.0 g L^{-1}, tartaric acid 4.4 g L^{-1}, shikimic acid 46 mg L^{-1}, acetic acid 0.12 g L^{-1}, citric acid 0.20 g L^{-1} and total amino acids (without proline) 1314.6 mg L^{-1} (**Table A-1**). Three days before the preparation of grape must, 30 mg L^{-1} of sulphur dioxide were added in grape juice as KMS. Experiments were performed in triplicate in 0.75-liter bottles filled with 650 ml of grape juice without yeast nutrient addition. Thirteen commercial *Saccharomyces* yeast strains (EC1118, Sauvignon, VL3, X5, X16, VIN13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2 and QA23) were used in this study. The yeast cultures were rehydrated following the method described in **3.2** prior to inoculation. The bottles were fitted with airlocks that enable the carbon dioxide to escape then the fermentations were carried out at 20 ^{o}C in an incubator. The consequent methods were performed as described in **3.7.1**. Then 150 ml wine samples were kept at -18 ^{o}C for the analysis as describe in method **3.7.1**.

The one way ANOVA and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

3.8 Fermentation trials of Sauvignon blanc grape juice

The fermentation trials of Sauvignon blanc grape juice were performed in two sequential trials as follows.

3.8.1 Fermentation of fresh grape juice with nine commercial *Saccharomyces* yeast strains and two nutrient sources

The fermentation was carried out in fresh Sauvignon blanc grape juice from the 2008 harvest season. Nine commercial *Saccharomyces* yeast strains and two commercial nutrient sources were used in this study, thus giving 18 different fermentation treatments (**Table 3-8)**. Properties of initial grape juice were pH 3.10, total soluble solid 17.2 ^{o}Brix, reducing sugar content 169 g L^{-1}, total acidity 10.1 g L^{-1}, tartaric acid 6.8 g L^{-1}, malic acid 7.0 g L^{-1}, shikimic acid 38 mg L^{-1}, citric acid 0.3 g L^{-1}, glycerol 0.3 g L^{-1}, ammonium 0.08 g L^{-1}, free alpha amino nitrogen 92.5 mg L^{-1} and total amino acids (without proline) 1264.6 mg L^{-1} (**Table A-2**). The experimental fermentations were carried out in 0.75-liter bottles containing 650 ml grape

juice and all treatments were done in duplicate. Addition of nutrients was performed in the grape juice according to an experimental design consisting of two different nutrient sources prior to the alcoholic fermentation (**Table 3-8)**. Then 25 mg L^{-1} of sulphur dioxide were added into the grape juice by addition of KMS, and juice bottles were left approximately 12 hours before inoculation. The yeast cultures were rehydrated following the preparation described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The following methods were performed as described before in **3.7.1**. Consequently, 150 ml wine samples were kept at -18 ^{o}C for the analysis as described in method **3.7.1**. The experimental design is shown in **Table 3-8**.

The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-8 Experimental design of yeast inoculation and nutrient supplementation in fresh Sauvignon blanc grape juice from the 2008 harvest season

Saccharomyces yeast strain	Initial nutrient source (0.3 g L^{-1})
EC1118	Fermaid E
	OptiWhite
Sauvignon	Fermaid E
	OptiWhite
VL3	Fermaid E
	OptiWhite
X5	Fermaid E
	OptiWhite
VIN7	Fermaid E
	OptiWhite
VIN13	Fermaid E
	OptiWhite
4F9	Fermaid E
	OptiWhite
LVCB	Fermaid E
	OptiWhite
AWRI R2	Fermaid E
	OptiWhite

3.8.2 Fermentation of fresh grape juice with five commercial *Saccharomyces* yeast strains and four nutrient sources

The fermentation was carried out in fresh Sauvignon blanc grape juice from the 2009 harvest season. The properties of initial grape juice were total soluble solid 17.3 ^{o}Brix, reducing sugar content 190 g L^{-1}, pH 3.19, free sulphur dioxide 2.8 mg L^{-1}, tartaric acid 5.1 g L^{-1}, malic acid 4.0 g L^{-1}, shikimic acid 22 mg L^{-1}, citric acid 0.2 g L^{-1}, glycerol 0.50 g L^{-1} , NOPA 164 mg L^{-1} (as isoleucine) and total amino acids (without proline) 1782.9 mg L^{-1} (**Table A-2**). Five commercial *Saccharomyces* yeast strains and four commercial nutrient sources were used in this study, thus giving 20 different fermentation treatments (**Table 3-9)**. Triplicate experimental fermentations were carried out in 0.75-liter bottles containing 620 ml grape juice. Addition of nutrient was performed in the grape juice according to an experimental design prior to the alcoholic fermentation. Then 25 mg L^{-1} of sulphur dioxide was added in grape juice as KMS, and juice bottles were left approximately 12 hours before inoculation. The yeast cultures were rehydrated following the method described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The consequent methods were performed as described in **3.7.1**. In addition, 150 ml wine samples were kept at -18 ^{o}C for the analysis of sulphur dioxide produced by yeast and the other analysis as described in method **3.7.1**. The experimental design is shown in **Table 3-9**.

The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-9 Experimental design of yeast inoculation and nutrient supplementation in Sauvignon blanc grape juice fermentation

Saccharomyces yeast strain	Initial nutrient source
EC1118	Superstart 0.3 g L^{-1}
	Fermaid O 0.4 g L^{-1}
	Fermaid E 0.4 g L^{-1}
	VUltra 0.4 g L^{-1}
X5	Superstart 0.3 g L^{-1}
	Fermaid O 0.4 g L^{-1}
	Fermaid E 0.4 g L^{-1}
	VUltra 0.4 g L^{-1}
VIN7	Superstart 0.3 g L^{-1}
	Fermaid O 0.4 g L^{-1}
	Fermaid E 0.4 g L^{-1}
	VUltra 0.4 g L^{-1}
Alchemy I	Superstart 0.3 g L^{-1}
	Fermaid O 0.4 g L^{-1}
	Fermaid E 0.4 g L^{-1}
	VUltra 0.4 g L^{-1}
LittoLevure	Superstart 0.3 g L^{-1}
	Fermaid O 0.4 g L^{-1}
	Fermaid E 0.4 g L^{-1}
	VUltra 0.4 g L^{-1}

3.9 Fermentation trials of yellow passion fruit juice

The fermentation trials of yellow passion fruit (*Passiflora edulis* Sims *f. flavicarpa* Degner) juices obtained from frozen yellow passion fruit puree were performed in four sequential trials as follows.

3.9.1 Influence of fourteen different commercial *Saccharomyces* yeast strains on fermentation characteristics and quality of yellow passion fruit wine

The frozen yellow passion fruit (YPF) puree was used, and its properties are reducing sugar content 61.5 g L^{-1}, pH 2.8, NOPA 332 mg L^{-1} (as isoleucine), malic acid 5.4 g L^{-1}, shikimic acid 14 mg L^{-1}, citric acid 38.4 g L^{-1} and total amino acid without proline 2491.0 mg L^{-1} (**Table A-3**). The frozen YPF puree was thawed at ambient temperature and diluted by addition of hot water until its pH was 3.1-3.2, therefore the reducing sugar content of diluted YPF juice was 8.1 g L^{-1}, NOPA was 46 mg L^{-1} (as isoleucine) and total amino acids (without proline) were 207.1 mg L^{-1} (**Table A-3**). The sugar content was adjusted to provide sugar quantities of 200 g Kg^{-1} juice by addition of beet sugar (sucrose, Südzucker, Mannheim, Germany), and 0.5 g L^{-1} DAP was added for nutrient supplementation. The composition of prepared YPF juice was inverted sugar content 207 g L^{-1}, pH 3.22, malic acid 0.5 g L^{-1}, citric acid 3.7 g L^{-1} and shikimic acid 1 mg L^{-1}. Triplicate experiments were performed in 0.75-liter bottles filled with 650 ml YPF juice, and 50 mg L^{-1} of sulphur dioxide as KMS was added. Juice bottles were left approximately 12 hours to suppress undesirable microorganism growth as well as to function as an antioxidant. Fourteen commercial *Saccharomyces* yeast strains (EC1118, Sauvignon, VL3, X5, X16, VIN13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2, QA23 and Freddo) were used in this study. The yeast cultures were rehydrated following the method described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks that enable the carbon dioxide to escape then the fermentations were carried out at 20 ^{o}C in a controlled incubator. The progress of fermentation was followed by monitoring CO_2 production, which was determined by weight loss during fermentation. After the weight loss of the samples were constant, YPF wines were cold stabilized at below 10 ^{o}C for 7 days and racked into previously cleaned bottles. Then 80 mg L^{-1} of SO_2 was added as KMS in finished YPF wine and bottled wines were stored at below 15 ^{o}C until need. Then 150 ml YPF wines without sulphur dioxide addition, were kept at -18 ^{o}C for analysis of SO_2-binding compounds, glycerol and residual sugar.

The one way ANOVA and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

3.9.2 Fermentation of yellow passion fruit juice with three commercial *Saccharomyces* yeast strains and five commercial nutrient sources

The properties of frozen yellow passion fruit puree used in this study are reducing sugar content 67.1 g L^{-1}, pH 2.77, NOPA 317 mg L^{-1} (as isoleucine), malic acid 5.9 g L^{-1}, citric acid 41.3 g L^{-1} and total amino acid without proline 2491.0 mg L^{-1} (**Table A-3**). The frozen YPF puree was thawed at ambient temperature and diluted by addition of hot water until its pH was 3.1-3.2, then reducing sugar content of diluted YPF juice was 10.7 g L^{-1}, NOPA was 43 mg L^{-1} (as isoleucine) and total amino acids (without proline) were 180.3 mg L^{-1} (**Table A-3**). Then the sugar content was adjusted to provide sugar quantities of 200 g Kg^{-1} juice by addition of beet sugar (sucrose, Südzucker, Mannheim, Germany). The composition of prepared YPF juice was inverted sugar content 201 gL^{-1}, pH 3.2, malic acid 0.5 g L^{-1} and citric acid 3.8 g L^{-1}. Triplicate experiments were performed in the 1.50-liter bottles filled with 1.20 L YPF juice. Three commercial *Saccharomyces* yeast strains and five commercial nutrient sources were used in this study, thus giving 15 different fermentation treatments (**Table 3-10)**. Addition of nutrients was performed in the YPF juice according to an experimental design prior to the alcoholic fermentation and 50 mg L^{-1} of sulphur dioxide as KMS was added. Juice bottles were left approximately 12 hours to suppress undesirable microorganism growth as well as to function as an antioxidant. The yeast cultures were rehydrated following the method described in **3.2** prior to the inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The consequent methods were performed as described in **3.9.1**. Then 150 ml of YPF wine samples were kept at -18 ^{o}C for the analysis as described in method **3.9.1**. The experimental plan is shown in **Table 3-10**.

The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-10 Experimental design of yeast inoculation and nutrient supplementation in prepared YPF juice fermentation

Saccharomyces yeast strain	Initial nutrient source (0.4 g L^{-1})
EC1118	DAP
	VCombi
	VUltra
	Fermaid E
	OptiWhite
Sauvignon	DAP
	VCombi
	VUltra
	Fermaid E
	OptiWhite
X5	DAP
	VCombi
	VUltra
	Fermaid E
	OptiWhite

3.9.3 Fermentation of yellow passion fruit juice with five commercial *Saccharomyces* yeast strains and two commercial nutrient sources at two different concentrations

The frozen yellow passion fruit puree was used in this study, and its composition was reducing sugar content 50.5 g L^{-1}, pH 2.89, NOPA 296 mg L^{-1} (as isoleucine), malic acid 5.2 g L^{-1}, citric acid 40.5 g L^{-1} and total amino acid without proline 2491.0 mg L^{-1} (**Table A-3**). The frozen YPF puree was thawed at ambient temperature and diluted by addition of hot water until its pH was 3.1-3.2, therefore reducing sugar content of diluted YPF juice was 3.65 g L^{-1}, NOPA was 24 mg L^{-1} (as isoleucine) and total amino acids (without proline) 123.1 mg L^{-1} (**Table 4-23** and **A-3**). Then the sugar content was adjusted to provide sugar quantities of 200 g Kg^{-1} juice by addition of beet sugar (sucrose, Südzucker, Mannheim, Germany). The composition of prepared YPF juice was inverted sugar content 170.5 g L^{-1}, pH 3.19, malic acid 0.5 g L^{-1} and citric acid 3.1 g L^{-1}. Experiments were performed in triplicate in 1.50-liter bottles filled with 1.20 L YPF juice. Five commercial *Saccharomyces* yeast strains and two commercial nutrient sources at two different concentrations were used in this study, thus giving 20 different fermentation treatments (**Table 3-11)**. Addition of nutrients was

performed in the YPF juice according to an experimental design (**Table 3-11)** prior to the alcoholic fermentation and 50 mg L^{-1} of sulphur dioxide as KMS were added. Juice bottles were left to settle approximately 12 hours and the yeast cultures were rehydrated following the method described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The consequent methods were performed as described in **3.9.1**. 150 ml of YPF wine samples were kept at -18 ^{o}C for the analysis as described in method **3.9.1**. The experimental design is shown in **Table 3-11**.

The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-11 Experimental design of yeast inoculation and nutrient supplementation in prepared YPF juice fermentation

Saccharomyces yeast strain	Initial nutrient source	Nutrient concentration (g L^{-1})
EC1118	DAP	0.25
		0.50
	VCombi	0.25
		0.50
VL3	DAP	0.25
		0.50
	VCombi	0.25
		0.50
X5	DAP	0.25
		0.50
	VCombi	0.25
		0.50
Alchemy I	DAP	0.25
		0.50
	VCombi	0.25
		0.50
LittoLevure	DAP	0.25
		0.50
	VCombi	0.25
		0.50

3.9.4 Fermentation of yellow passion fruit juice with three commercial *Saccharomyces* yeast strains and four commercial nutrient sources at two different concentrations

The properties of frozen yellow passion fruit puree used in this study were reducing sugar content 43 gL^{-1}, pH 2.85, NOPA 296 mg L^{-1} (as isoleucine), malic acid 5.3 g L^{-1}, citric acid 42.1 g L^{-1} and total amino acid without proline 2491.0 mg L^{-1} (**Table A-3**). The frozen YPF puree was thawed at ambient temperature and diluted by addition of hot water until its pH was 3.1-3.2, thus giving the reducing sugar content of diluted YPF juice of 4 g L^{-1}, NOPA was 27 mg L^{-1} (as isoleucine) and total amino acids (without proline) 164.2 mg L^{-1} (**Table 4-28** and **A-3**). Then the sugar content was adjusted to provide sugar quantities of 200 g Kg^{-1} juice by addition of beet sugar (sucrose, Südzucker, Mannheim, Germany). The composition of prepared YPF juice was inverted sugar content 170 g L^{-1}, pH 3.18, malic acid 0.5 g L^{-1} and citric acid 2.8 g L^{-1}. Triplicate experiments were performed in 0.75-liter bottles filled with 650 ml YPF juice. Three commercial *Saccharomyces* yeast strains and four commercial nutrient sources at two different concentrations were used in this study, thus giving 24 different fermentation treatments (**Table 3-12)**. Addition of commercial nutrients was performed in the YPF juice according to an experimental design (**Table 3-12)** prior to the alcoholic fermentation and 50 mg L^{-1} of sulphur dioxide as KMS were added. Juice bottles were left approximately 12 hours to suppress undesirable microorganism growth as well as to function as an antioxidant. The yeast cultures were rehydrated following the preparation of yeast culture described in **3.2** prior to inoculation of each strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 ^{o}C in a controlled environment. The following methods were performed as described in **3.9.1**. In addition, 150 ml of YPF wine samples were kept at -18 ^{o}C for the analysis as described in method **3.9.1**. The experimental plan is shown in **Table 3-12**.

The ANOVA for factorial design and DMRT were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm, 1994) to interpret mean differences in mean values at the 95 % confidence level.

Table 3-12 Experimental design of yeast inoculation and nutrient supplementation in prepared YPF juice fermentation

Saccharomyces yeast strain	Initial nutrient source	Nutrient concentration (g L^{-1})
EC1118	VCombi	0.2
		0.4
	VUltra	0.2
		0.4
	Fermaid E	0.2
		0.4
	OptiWhite	0.2
		0.4
VL3	VCombi	0.2
		0.4
	VUltra	0.2
		0.4
	Fermaid E	0.2
		0.4
	OptiWhite	0.2
		0.4
X5	VCombi	0.2
		0.4
	VUltra	0.2
		0.4
	Fermaid E	0.2
		0.4
	OptiWhite	0.2
		0.4

Investigation of volatile thiols in yellow passion fruit wine

YPF wine samples obtained from the fermentation trials of **3.9.3** and **3.9.4** were selected to investigate for volatile thiols in finished products after fermentation. The volatile thiols in YPF wine were specifically extracted by the reversible combination of the thiols with ρ-hydroxymercuribenzoate according to a modified Ferreira et al. (2007) method and analyzed by gas chromatography/mass spectrophotometry (GC-MS) within 24 hours as described in the method of **3.6.3.3 Volatile thiols involved in varietal aromas**.

4. RESULTS

The following chapters give an overview on the conducted experiments and the obtained results of each fermentation trial (see experimental outline for all fermentation trials in **Figure A-2**). Firstly, investigations on the effects of commercial *Saccharomyces* strains and nutrient supplements on wine fermentative characteristics and quality of Scheurebe grape wines are demonstrated in **chapter 4.1**. Secondly, the obtained results of the growth kinetics, metabolic products and wine aromas of Sauvignon blanc grape juice fermented with different commercial *Saccharomyces* yeast strains and nutrient supplements are presented in **chapter 4.2**. The improvement of fermentation kinetics, fermentative characteristics and quality of yellow passion fruit wine by optimal choice of commercial *Saccharomyces* yeast strains and nutrient supplements are reported in **chapter 4.3**. Finally, the results of the volatile thiols involved in varietal aromas of finished YPF wine obtained from the experiment **3.9.3** and **3.9.4** will be reported in **chapter 4.4**. Each experiment was carried out in duplicate or triplicate depending on the experimental plan.

4.1 Effects of commercial *Sacchromyces* yeast strains and nutrient supplements on wine fermentative characteristics and quality of Scheurebe grape wines

The two experimental results that are described in the following chapters were obtained from Scheurebe grape juice fermented with different commercial *Saccharomyces* yeast strains and/or nutrient sources at different concentrations.

4.1.1 Effect of two commercial *Saccharomyces* yeast strains and four nutrient sources on fermentative characteristics, metabolic compounds and wine aromas of Scheurebe grape wines

Fermentation was performed in fresh Scheurebe grape juices from the 2008 harvest season. Two commercial yeast strains, EC1118 and X5, were used to ferment grape juice supplemented with four different nutrient sources; OptiWhite (natural inactivated yeast), Superstart (blend of inactivated yeast and yeast cell walls), DAP and Fermaid E (blend of diammonium hydrogen phosphate, thiamine, yeast cell walls and ammonium sulphate), and no nutrient addition (control). The fermentation kinetics, some metabolic compounds and wine aroma in finished wines were examined.

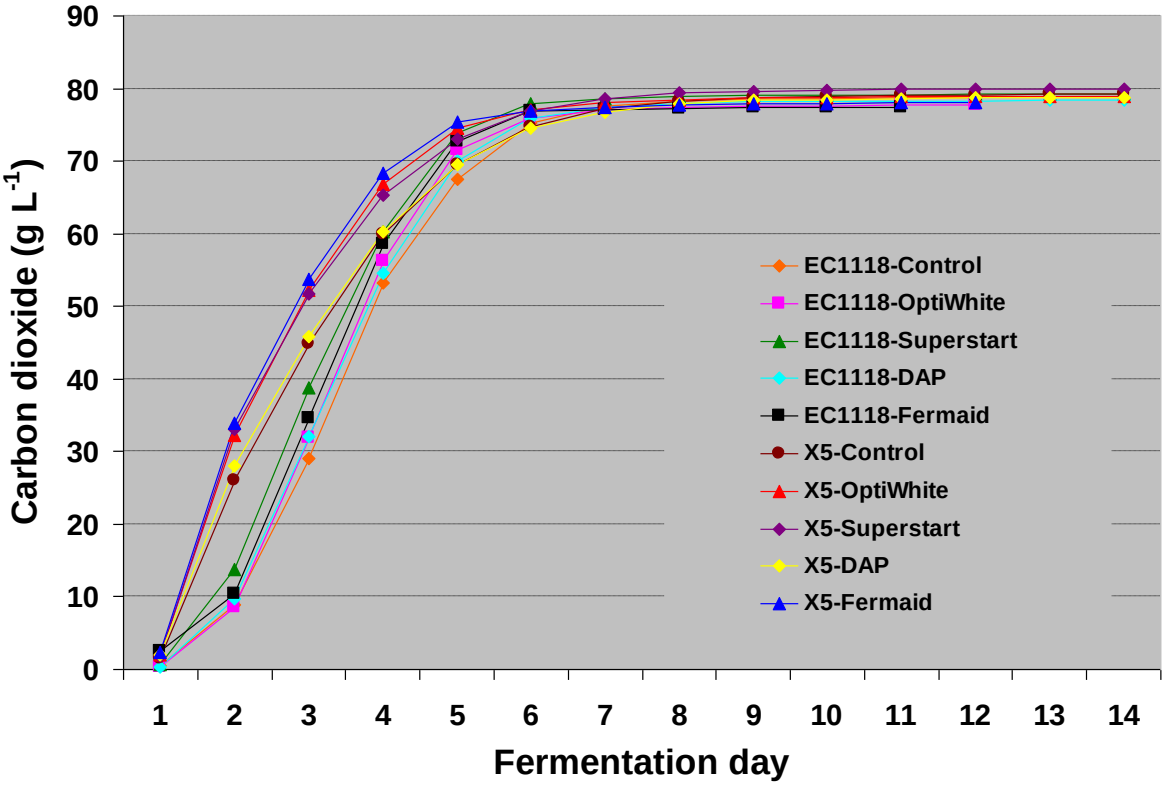


Figure 4-1 Growth kinetics of fresh Scheurebe grape juice fermentations with two yeast strains and four nutrient sources

The growth kinetics: The result in **Figure 4-1** shows that after the inoculation of the EC1118 and X5 strains into fresh Scheurebe grape juices, the growth kinetics of the yeast strains varied depending on the sources of nutrients added. The EC1118 strain seemed to have a slightly longer lag phase than the X5 strain, but the addition of Fermaid E reduced the fermentation time from 14 days to 11 days. The addition of Fermaid E also stimulated the X5 strain to have faster growth kinetics and fermentation time (12 days). All treatments completed fermentation after 11-14 days depending on the nutrient source added.

Chemical composition: **Figure 4-2** shows that significant differences existed between the treatment combinations in term of residual sugar in final wines, but not in glycerol production. Without nutrient addition to the juice (control), the EC1118 strain produced wine having high residual sugar (1.2 $\pm$ 0.0 g L^{-1}). Whereas, fermentation with strain X5 in the presence of Superstart resulted in the highest residual sugar in the final wine (1.0 $\pm$ 0.0 g L^{-1}). Addition of any kind of nutrients led to decreased residual sugar in the final wine. Nevertheless, residual sugar content in all wine treatments was below 2 g L^{-1} and the wines reached dryness. The two yeast strains produced similar amounts of glycerol in wines treated with any source of nutrient and in the control treatment ranging from 2.5 $\pm$ 0.1 to 2.8 $\pm$ 0.0 g L^{-1}.

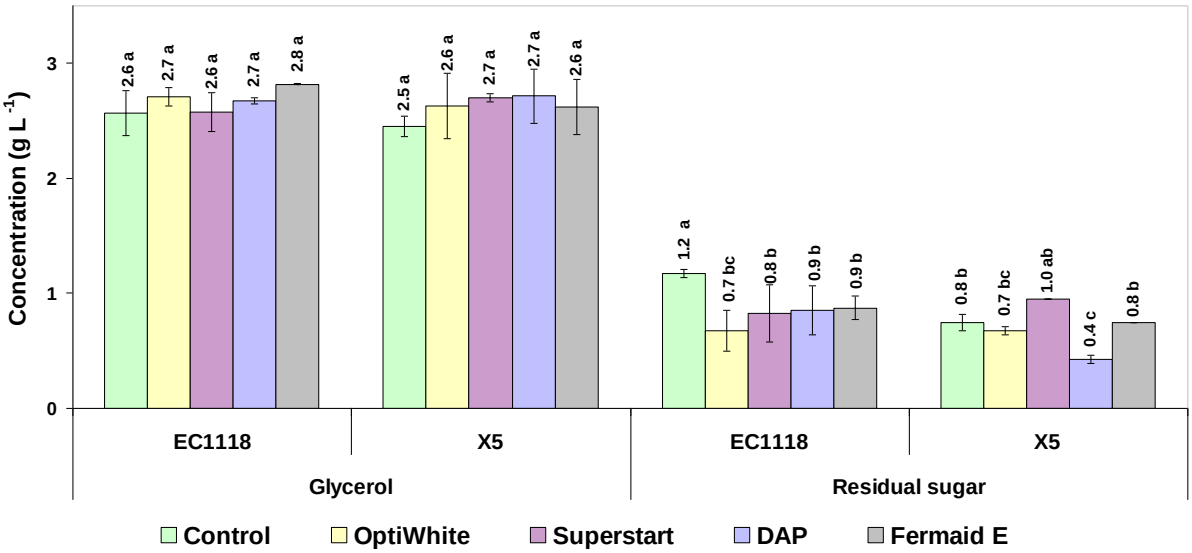


Figure 4-2 Concentration of residual sugar and glycerol in finished Scheurebe grape wines fermented with two yeast strains, four nutrient sources and without nutrient

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each compound, whereas means followed by the same letters are not significantly different ($p>0.05$) according to the DMRT test.

Organic acids: **Table 4-1** shows the concentration of organic acids found in obtained wines fermented with strains EC1118 and X5 with different nutrient sources and without nutrients. The results indicate that both yeast strains and nutrient sources only affected the production of acetic acid, while the other organic acids were similar among wine treatments. The two yeast strains produced high concentrations of acetic acid in the control treatment (0.29 $\pm$ 0.00 and 0.29 $\pm$ 0.01 g L^{-1}, respectively). However, when a nutrient was added they had inverse patterns of acetic acid formation. The presence of OptiWhite stimulated the highest formation of this acid by strain EC1118 (0.29 $\pm$ 0.00 g L^{-1}), while strain X5 produced the highest amounts in the Superstart treatment (0.27 $\pm$ 0.00 g L^{-1}). The other organic acid concentrations in all wine treatments showed no difference ranging from 1.16 $\pm$ 0.22 to 1.41 $\pm$ 0.44 g L^{-1} tartaric acid, 5.04 $\pm$ 0.17 to 5.25 $\pm$ 0.35 g L^{-1} malic acid, 0.12 $\pm$ 0.00 to 0.17 $\pm$ 0.01 g L^{-1} lactic acid, 45.67 $\pm$ 0.02 to 47.03 $\pm$ 0.83 g L^{-1} shikimic acid 0.21 $\pm$ 0.00 to 0.28 $\pm$ 0.02 g L^{-1} citric acid.

Table 4-1 Concentration of organic acids found in finished Scheurebe wines developed by two yeast strains with four nutrient sources and without nutrient

Yeast	Nutrient	Tartaric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Lactic acid ($g\ L^{-1}$)	Shikimic acid ($mg\ L^{-1}$)	Acetic acid ($g\ L^{-1}$)	Citric acid ($g\ L^{-1}$)
EC1118	Control	1.19 ± 0.13 a	5.22 ± 0.18 a	0.12 ± 0.00 a	47.03 ± 0.83 a	0.29 ± 0.00 a	0.24 ± 0.03 a
	OptiWhite	1.29 ± 0.25 a	5.07 ± 0.10 a	0.13 ± 0.01 a	46.06 ± 1.09 a	0.29 ± 0.00 a	0.23 ± 0.03 a
	Superstart	1.30 ± 0.16 a	5.15 ± 0.05 a	0.14 ± 0.00 a	46.51 ± 0.15 a	0.22 ± 0.02 cd	0.23 ± 0.03 a
	DAP	1.16 ± 0.22 a	5.24 ± 0.03 a	0.15 ± 0.01 a	46.18 ± 0.19 a	0.23 ± 0.01 cd	0.23 ± 0.03 a
	Fermaid E	1.23 ± 0.31 a	5.07 ± 0.06 a	0.14 ± 0.01 a	46.41 ± 0.03 a	0.25 ± 0.02 bc	0.26 ± 0.03 a
X5	Control	1.16 ± 0.23 a	5.06 ± 0.10 a	0.12 ± 0.02 a	45.67 ± 0.02 a	0.29 ± 0.01 a	0.21 ± 0.00 a
	OptiWhite	1.25 ± 0.16 a	5.04 ± 0.17 a	0.12 ± 0.01 a	46.46 ± 1.23 a	0.23 ± 0.02 cd	0.25 ± 0.05 a
	Superstart	1.17 ± 0.11 a	5.25 ± 0.35 a	0.13 ± 0.00 a	47.01 ± 0.65 a	0.12 ± 0.02 e	0.28 ± 0.02 a
	DAP	1.41 ± 0.44 a	5.05 ± 0.00 a	0.14 ± 0.02 a	46.39 ± 0.11 a	0.27 ± 0.00 ab	0.23 ± 0.01 a
	Fermaid E	1.29 ± 0.19 a	5.18 ± 0.00 a	0.17 ± 0.01 a	45.87 ± 0.26 a	0.20 ± 0.04 d	0.21 ± 0.02 a

Each value shows the mean ± standard deviation.

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

SO_2-binding compounds: As shown in **Figure 4-3**, the results show that different production of these compounds by the two yeast strains varied depending on the nutrient sources, excluding acetaldehyde. Regarding α-ketoglutarate, strain EC1118 produced the highest amounts in the Superstart treatment (26.3 ± 0.5 mg L^{-1}), while the highest production by strain X5 was present in the OptiWhite, Superstart and DAP treatments (23.3 ± 3.4, 23.6 ± 1.8 and 24.0 ± 2.6 mg L^{-1}, respectively). Straine X5 was the low pyruvate producer (7.8 ± 2.4 to 15.4 ± 0.7 mg L^{-1}). On the contrary, strain EC1118 was the high pyruvate producer (20.8 ± 1.0 to 27.6 ± 1.3 mg L^{-1}), but its formation tended to decrease with the addition of nutrients, particularly in the Fermaid E treatment. These results show that the addition of Fermaid E significantly reduced the formation of these keto acids. Levels of acetaldehyde were similar among wine treatments ranging from 38.4 ± 0.2 to 48.5 ± 2.0 mg L^{-1}.

Sulphur containing compounds: The data in **Figure 4-4** indicate wines produced by strain EC1118 did not reveal H_2S under this trial condition, but the X5 strain produced high amounts, particularly in the control and Superstart treatment (2.9 ± 0.1 and 3.0 ± 0.2 μg L^{-1}, respectively). There was no statistical difference of dimethyl sulphide formations among treatment combinations ranging from 1.1 ± 0.1 to 1.9 ± 0.5 μg L^{-1}. Under this trial condition, the other sulphur compounds like ethanethiol, methanethiol, dimethyl disulphide, thioacetic acid S-methylester and thioacetic acid S-ethylester were not detected in all wine treatments.

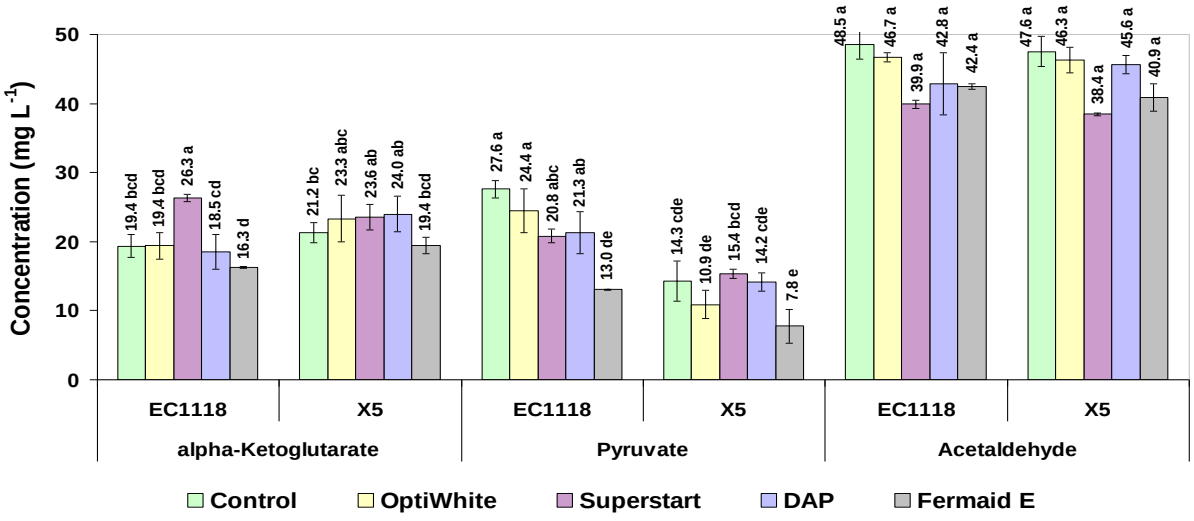


Figure 4-3 Concentration of SO_2-binding compounds in finished Scheurebe grape wines produced by two yeast strains with four nutrient sources and without nutrient

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each S-binding compound, whereas means followed by the same letter are not significantly different ($p>0.05$) according to the DMRT test.

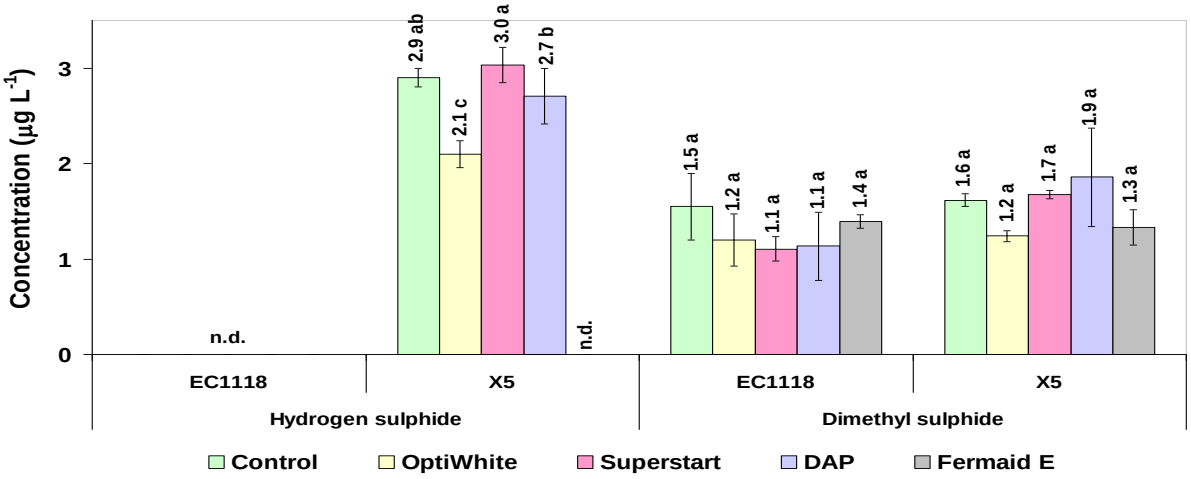


Figure 4-4 Formation of hydrogen sulphide and dimethyl sulphide in finished Scheurebe grape wines produced by two yeast strains with four nutrient sources and without nutrient

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each S-containing compound, whereas means followed by the same letter are not significantly different ($p>0.05$) according to the DMRT test.

Higher alcohols: Commercial yeast strains and nutrient sources significantly affected only 2-methyl propanol and 3-methyl butanol in the final wines (**Table 4-2**). The EC1118 strain seemed to produce higher concentrations of these alcohols with less response to the nutrient source than the X5 strain. However, 2-methyl propanol seemed to be most evident in the Superstart and DAP treatments (24.0 $\pm$ 1.4 and 24.0 $\pm$ 3.2 mg L^{-1}, respectively), whereas 3-methyl butanol seemed to be the highest in the Feramid E treatment (164.0 $\pm$ 5.1 mg L^{-1}). On the other hand, strain X5 developed the highest amounts of two alcohols in the Fermaid E treatment (22.5 $\pm$ 2.1 and 156.5 $\pm$ 2.3 mg L^{-1}, respectively). There were no significant differences among wine treatments in respect to 2-methyl butanol and hexanol formations ranging from 19.0 $\pm$ 1.2 to 27.0 $\pm$ 0.9 mg L^{-1} and 1627.5 $\pm$ 161.9 to 2062.0 $\pm$ 9.3 µg L^{-1}, respectively. **Figure 4-5** shows that both yeast strains and nutrient sources had no significant effects on the production of 2-phenyl ethanol ranging from 16.4 $\pm$ 1.0 to 24.4 $\pm$ 1.4 mg L^{-1}.

Table 4-2 Concentration of higher alcohols found in finished Scheurebe wines developed by two yeast strains with four nutrient sources and without nutrient

Yeast	Nutrient	2-Methyl propanol (mg L^{-1})	Hexanol (μg L^{-1})	3-Methyl butanol (mg L^{-1})	2-Methyl butanol (mg L^{-1})
EC1118	Control	22.5 ± 3.9 ab	1991.0 ± 83.1 a	134.0 ± 12.5 bcd	20.5 ± 2.2 a
	OptiWhite	20.0 ± 1.2 abc	2011.5 ± 116.8 a	132.5 ± 6.1 bcd	21.0 ± 1.3 a
	Superstart	24.0 ± 1.4 a	1972.0 ± 10.0 a	150.5 ± 9.6 ab	24.0 ± 1.3 a
	DAP	24.0 ± 3.2 a	2062.0 ± 9.3 a	142.0 ± 12.7 abc	21.0 ± 1.5 a
	Fermaid E	18.5 ± 2.4 abc	2039.0 ± 137.2 a	164.0 ± 5.1 a	23.0 ± 1.4 a
X5	Control	17.0 ± 1.5 bc	1826.5 ± 109.8 a	113.0 ± 9.1 de	21.0 ± 2.4 a
	OptiWhite	17.0 ± 2.4 bc	1778.5 ± 2.7 a	110.0 ± 7.1 de	21.5 ± 2.4 a
	Superstart	21.0 ± 5.8 abc	1627.5 ± 161.9 a	117.0 ± 23.8 cde	22.5 ± 5.1 a
	DAP	15.5 ± 0.9 c	1808.5 ± 126.5 a	105.0 ± 6.6 e	19.0 ± 1.2 a
	Fermaid E	22.5 ± 2.1 ab	1913.0 ± 10.8 a	156.5 ± 2.3 ab	27.0 ± 0.9 a

Each value shows the mean ± standard deviation.

Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

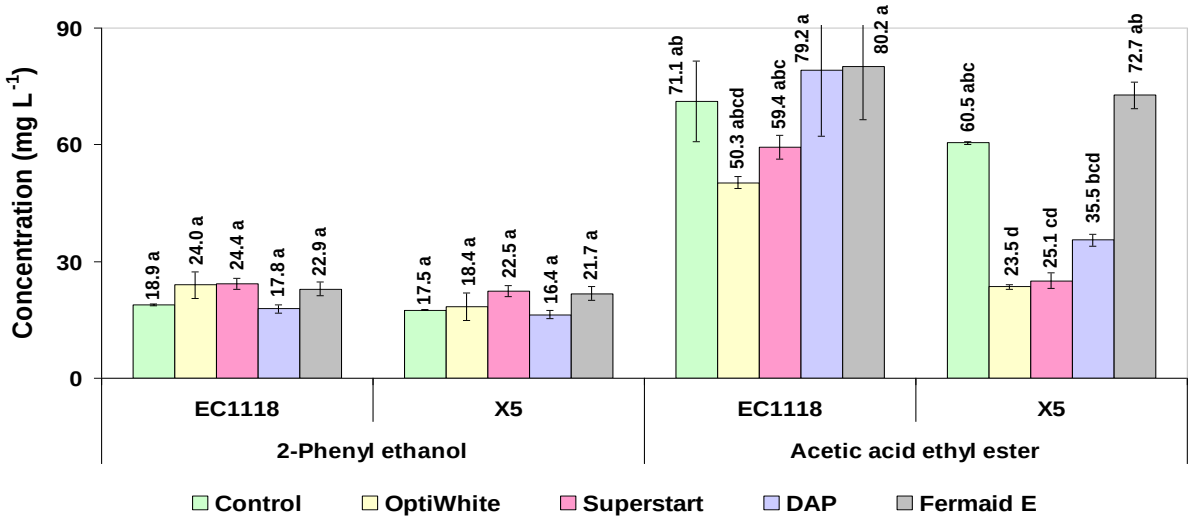


Figure 4-5 Formation of 2-phenyl ethanol and acetic acid ethyl ester in finished Scheurebe grape wines developed by two yeast strains with four nutrient sources and without nutrient

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each compound, whereas means followed by the same letters are not significantly different ($p>0.05$) according to the DMRT test.

Acetic acid esters: As shown in **Figure 4-5**, the EC1118 strain seemed to produce higher amounts of acetic acid ethyl ester (50.3 ± 1.5 and 80.2 ± 13.7 mg L^{-1}) than the X5 strain and was less response to the nutrient sources. In contrast, strain X5 developed high amounts of this ester in the Fermaid E and control treatments (72.7 ± 3.4 and 60.5 ± 0.3 mg L^{-1}). Regarding the other acetic acid esters, the two yeast strains produced different amounts of these esters depending on the nutrient source (**Figure 4-6**). Strain EC1118 seemed to produce the highest amount of acetic acid 3-methylbutyl ester in the Fermaid E sample (691.4 ± 38.7 μg L^{-1}), followed by the DAP, Superstart and control treatments (637.3 ± 135.9, 587.2 ± 6.9 and 532.5 ± 24.1 μg L^{-1}, respectively), and that of acetic acid 2-phenyl ethyl ester in the control and Fermaid E sample (111.7 ± 6.7 and 110.3 ± 4.5 μg L^{-1}, respectively). The X5 strain developed high amounts of these esters in the Superstart (571.4 ± 58.9 and 106.2 ± 0.9 μg L^{-1}, respectively) and Fermaid E treatments (744.9 ± 59.6 and 100.1 ± 14.0 μg L^{-1}, respectively). Nevertheless, addition of Fermaid E resulted in the highest formation of acetic acid 2-methylbutyl ester by both strains (30.6 ± 2.7 and 36.8 ± 4.2 μg L^{-1}, respectively). Both yeast strains produced similar amounts of acetic acid hexyl ester in wines ranging from 87.8 ± 14.9 to 110.3 ± 3.7 μg L^{-1}.

Ethyl and diethyl esters: As shown in **Table 4-3**, the production of ethyl and diethyl esters by the two strains varied significantly depending on the nitrogen sources, except isobutanoic acid ethyl ester (iBuEtE). Concentrations of iBuEtE were similar ranging from 39.0 ± 5.3 to 51.5 ± 4.4 μg L^{-1}. Strain EC1118 seemed to be the high producer of propionic acid ethyl ester (PrEtE), butanoic acid ethyl ester (BuEtE) and lactic acid ethyl ester (LAEtE) with less response to the nutrient source. The EC1118 wines had 100.0 ± 11.8 to 119.5 ± 6.1 μg L^{-1} PrEtE, 188.5 ± 12.5 to 239.5 ± 21.6 μg L^{-1} BuEtE and 22.5 ± 2.2 to 26.5 ± 4.6 mg L^{-1} LAEtE. Nevertheless, concentrations of these esters tended to be lower in the control and OptiWhite samples. The highest concentration of LAEtE was produced by strain X5 in the presence of Fermaid E (32.5 ± 8.0 mg L^{-1}), whereas PrEtE and BuEtE were most evident in the Fermaid E as well (92.0 ± 1.3 and 202.0 ± 28.3 μg L^{-1}, respectively). Regarding the succinic acid diethyl ester (SAdiEtE), the presence of Superstart promoted the highest formation of this ester by both yeast strains (1388.5 ± 240.2 and 1539.5 ± 41.8 μg L^{-1}, respectively).

Table 4-3 Concentration of ethyl esters and diethyl ester found in finished Scheurebe wines developed by two yeast strains with four nutrient sources and without nutrient

Yeast	Nutrient	PrEtE (μg L^{-1})	BuEtE (μg L^{-1})	iBuEtE (μg L^{-1})	LAEtE (mg L^{-1})	SAdiEtE (μg L^{-1})
EC1118	Control	100.0 ± 11.8 a	223.0 ± 18.5 abc	44.5 ± 4.8 a	22.5 ± 2.2 bc	818.0 ± 170.8 d
	OptiWhite	100.5 ± 0.7 ab	188.5 ± 12.5 bcd	41.5 ± 2.3 a	23.0 ± 1.8 bc	1149.0 ± 80.7 bc
	Superstart	111.5 ± 9.1 a	237.0 ± 5.7 ab	51.5 ± 4.4 a	25.5 ± 0.9 abc	1388.5 ± 240.2 ab
	DAP	119.5 ± 6.1 a	239.5 ± 21.6 a	47.5 ± 3.5 a	26.5 ± 4.6 ab	810.5 ± 12.2 d
	Fermaid E	114.5 ± 0.5 a	222.0 ± 5.4 abc	48.0 ± 1.4 a	25.5 ± 1.3 abc	846.5 ± 45.5 d
X5	Control	71.0 ± 13.7 c	162.5 ± 6.1 d	40.0 ± 7.1 a	18.5 ± 0.6 c	1311.5 ± 78.4 ab
	OptiWhite	76.0 ± 12.7 bc	164.5 ± 13.5 d	44.0 ± 6.5 a	20.0 ± 1.3 bc	1271.5 ± 16.3 b
	Superstart	65.5 ± 25.3 c	182.5 ± 39.1 cd	48.5 ± 13.4 a	21.5 ± 2.2 bc	1529.5 ± 41.8 a
	DAP	73.0 ± 12.7 bc	170.0 ± 26.9 d	39.0 ± 5.3 a	21.0 ± 0.5 bc	944.0 ± 93.1 cd
	Fermaid E	92.0 ± 1.3 abc	202.0 ± 28.3 abcd	50.5 ± 3.7 a	32.5 ± 8.0 a	1269.0 ± 4.2 b

Each value shows the mean ± standard deviation. Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

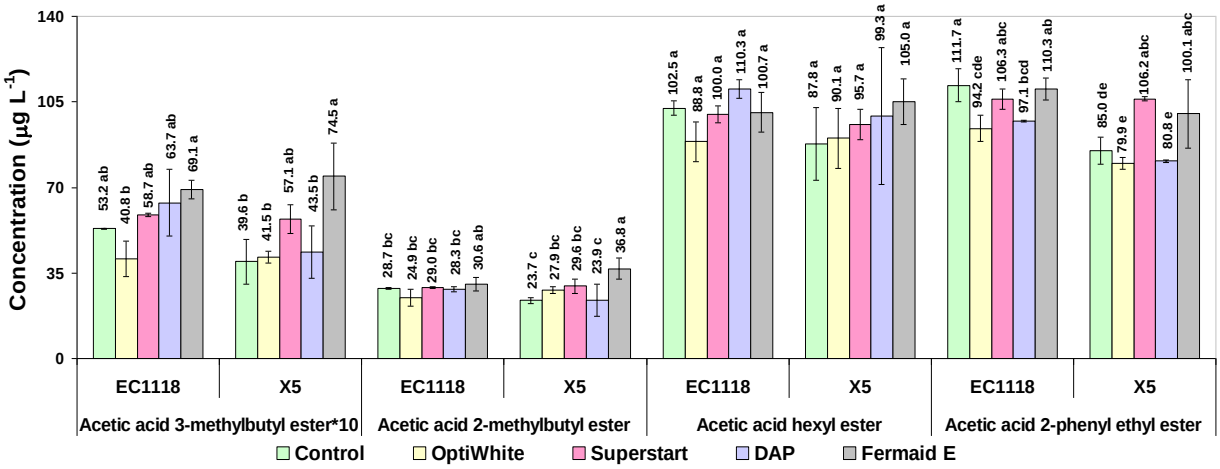


Figure 4-6 Formation of acetic acid esters in finished Scheurebe grape wines developed by two yeast strains with four nutrient sources and without nutrient (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each acetic acid ester, whereas means followed by the same letters are not significantly different ($p>0.05$) according to the DMRT test.

Medium-chain fatty acids (MCFAs) and their ethyl esters: **Table 4-4** shows that the yeast strain and the nutrient supplementation significantly affected the formation of hexanoic acid ethyl ester (HAEtE) only. The two yeast strains showed different patterns of HAEtE production in response to the nitrogen source addition. The EC1118 strain produced the highest amount of HAEtE in most treatments (685.5 $\pm$ 8.0, 690.5 $\pm$ 6.7, 714.5 $\pm$ 5.3 and 724.0 $\pm$ 9.6 μg L^{-1}, respectively), except in the OptiWhite sample. The wine treatments had similar concentrations of MCFAs ranging from 7.5 $\pm$ 0.7 to 9.5 $\pm$ 0.4 mg L^{-1} hexanoic acid, 7.5 $\pm$ 0.6 to 9.5 $\pm$ 0.3 mg L^{-1} octanoic acid and 1.6 $\pm$ 0.5 to 2.1 $\pm$ 0.2 mg L^{-1} decanoic acid. Concentrations of octanoic acid ethyl ester (OAEtE) and decanoic acid ethyl ester (DAEtE) were also similar among the wine samples that ranged from 1097.0 $\pm$ 121.5 to 1325.0 $\pm$ 1.3 μg L^{-1} and 383.0 $\pm$ 33.9 to 453.5 $\pm$ 19.0 μg L^{-1}, respectively.

Monoterpenes: As shown in **Figure 4-7**, the results show that the two yeast strains and nutrient sources did not affect the concentrations of all monoterpenes detected in wines. Nevertheless, all monoterpenes tended to slightly increase with the addition of any nutrient source. All wine treatments contained 124.4 $\pm$ 3.4 to 134.2 $\pm$ 4.0 μg L^{-1} of *trans*-linalool oxide, 30.2 $\pm$ 1.3 to 35.4 $\pm$ 1.7 μg L^{-1} of *cis*-linalool oxide, 34.2 $\pm$ 1.5 to 40.7 $\pm$ 3.0 μg L^{-1} of linalool and 70.0 $\pm$ 0.8 to 73.1 $\pm$ 0.2 μg L^{-1} of α-terpineol.

Table 4-4 Concentration of fatty acids and their esters found in finished Scheurebe wines developed by two yeast strains with four nutrient sources and without nutrient

Yeast	Nutrient	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})	HAEtE (μg L^{-1})	OAEtE (μg L^{-1})	DAEtE (μg L^{-1})
EC1118	Control	9.0 ± 0.2 a	9.0 ± 0.3 a	2.1 ± 0.2 a	685.5 ± 8.0 a	1200.5 ± 26.5 a	423.0 ± 28.4 a
	OptiWhite	8.5 ± 0.6 a	8.0 ± 0.0 a	1.7 ± 0.1 a	617.0 ± 16.7 b	1176.5 ± 30.6 a	386.0 ± 22.9 a
	Superstart	8.5 ± 0.6 a	8.5 ± 0.8 a	1.6 ± 0.5 a	690.5 ± 6.7 a	1238.5 ± 0.7 a	383.0 ± 33.9 a
	DAP	9.5 ± 0.4 a	9.5 ± 0.3 a	2.0 ± 0.0 a	714.5 ± 5.3 a	1272.5 ± 37.6 a	409.0 ± 16.6 a
	Fermaid E	9.0 ± 1.1 a	9.0 ± 1.1 a	1.8 ± 0.4 a	724.0 ± 9.6 a	1325.0 ± 1.3 a	428.5 ± 52.1 a
X5	Control	8.0 ± 0.2 a	8.0 ± 0.0 a	1.7 ± 0.1 a	547.0 ± 12.2 cd	1127.0 ± 2.8 a	453.5 ± 19.0 a
	OptiWhite	7.5 ± 0.2 a	8.0 ± 0.1 a	1.8 ± 0.2 a	531.0 ± 9.4 d	1097.0 ± 121.5 a	396.0 ± 35.8 a
	Superstart	8.0 ± 0.1 a	7.5 ± 0.6 a	1.7 ± 0.3 a	599.5 ± 0.6 bc	1225.0 ± 113.4 a	425.5 ± 5.9 a
	DAP	8.0 ± 0.2 a	8.5 ± 0.5 a	2.0 ± 0.1 a	563.5 ± 56.8 bcd	1123.5 ± 3.6 a	442.0 ± 8.4 a
	Fermaid E	8.0 ± 0.2 a	7.5 ± 2.2 a	1.6 ± 0.7 a	600.5 ± 41.7 bc	1189.0 ± 18.1 a	412.5 ± 31.6 a

Each value shows the mean ± standard deviation. Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

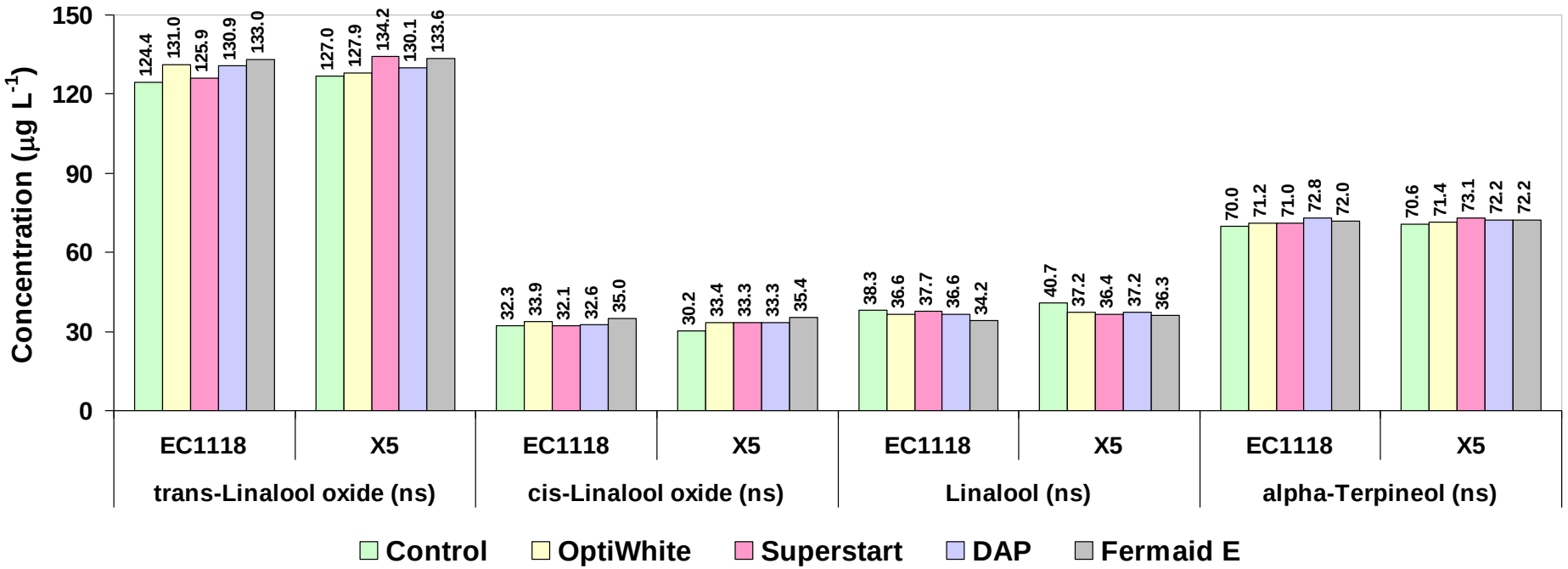


Figure 4-7 Formation of monoterpenes in finished Scheurebe grape wines developed by two yeast strains with four nutrient sources and without nutrient

Vertical bars represent standard deviations from two fermentation replicates. Monoterpenes displaying **"(ns)"** show no significant difference ($p>0.05$) between treatment combinations of each monoterpene according to the DMRT test.

4.1.2 Effect of thirteen commercial Saccharomyces yeast strains on fermentative characteristics, metabolic compounds and wine aromas of Scheurebe grape wines

Fermentation was performed in fresh Scheurebe grape juices from the 2008. Thirteen commercial yeast strains, EC1118, Sauvignon, VL3, X5, X16, VIN 13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2 and QA23 were used for the fermentation of this grape juice. The investigated results of the fermentation kinetics, some metabolic and aroma compounds of Scheurebe wines are described below.

Growth kinetics: The result in **Figure 4-8** shows significantly different growth kinetics of thirteen yeast fermentations. *Sacchromyces* strains EC1118, VIN13 and AWRI R2 were the strongest and fastest yeast fermenters (13 days) followed by VL3 and X5 (20 days), whereas the Alchemy II and QA23 strains were the slowest (35 days). The grape juice fermentations with strains Sauvignon, Alchemy I, 4F9, LVCB and LittoLevure resulted in stuck fermentations with long fermentation durations ranging from 25 to 35 days and high residual sugar concentrations (**Figure 4-9**), while the other strains successfully completed fermentations. Although the fermentation of strain QA23 was very slow, it was completed within 35 days with low residual sugar in the final wine.

Chemical compositions: **Figure 4-9** shows that the Sauvignon, LVCB and LittoLevure strains produced wine having the highest residual sugar (26.3 $\pm$ 3.3, 24.5 $\pm$ 3.5 and 25.6 $\pm$ 1.8 g L^{-1}, respectively), followed by strains 4F9 and Alchemy I. While, wines obtained from the other yeast strains had low amounts of residual sugar reaching dryness (1.2 $\pm$ 0.2 to 4.9 $\pm$ 0.5 g L^{-1}). There was a statistical difference for the glycerol production by the inoculated thirteen commercial yeast strains. The EC1118 and X16 strains were the highest glycerol producers (4.3 $\pm$ 0.1 and 4.4 $\pm$ 0.2 g L^{-1}, respectively) followed by strain VL3 (4.2 $\pm$ 0.1 g L^{-1}). In contrast, strains Sauvignon, 4F9 and LVCB were the lowest producers of this compound (3.6 $\pm$ 0.2, 3.7 $\pm$ 0.2 and 3.7 $\pm$ 0.1 g L^{-1}, respectively).

Organic acids: **Table 4-5** demonstrates that the wines produced by thirteen yeast strains had similar concentrations of tartaric, malic and shikimic acids which ranged from 0.85 $\pm$ 0.08 to 1.23 $\pm$ 0.30 g L^{-1}, 4.83 $\pm$ 0.16 to 5.23 $\pm$ 0.21 g L^{-1} and 42.79 $\pm$ 0.40 to 45.19 $\pm$ 1.38 mg L^{-1}, respectively. However, most yeast strains seemed to degrade approximately 13-20 % malic acid from 6.03 g L^{-1} in initial grape juice. The EC1118 strain produced the highest amounts of lactic acid (0.14 $\pm$ 0.00 g L^{-1}) followed by the AWRI R2, QA23 and VIN13 strains (0.12 $\pm$ 0.00, 0.10 $\pm$ 0.02 and 0.10 $\pm$ 0.01 g L^{-1}, respectively), whereas strain X16 produced the lowest

amount (0.06 ± 0.01 g L^{-1}). On the other hand, strain EC1118 and strain VIN13 produced lower amount of acetic acid (0.24 ± 0.01 and 0.20 ± 0.02 g L^{-1}) than the other strains. Regarding citric acid, its level was most evident in the VL3 and QA23 wines (0.25 ± 0.02 and 0.25 ± 0.06 g L^{-1}, respectively) followed by strains EC1118 and X16 (0.22 ± 0.02 and 0.22 ± 0.01 g L^{-1}, respectively), while the other wines had similar low levels.

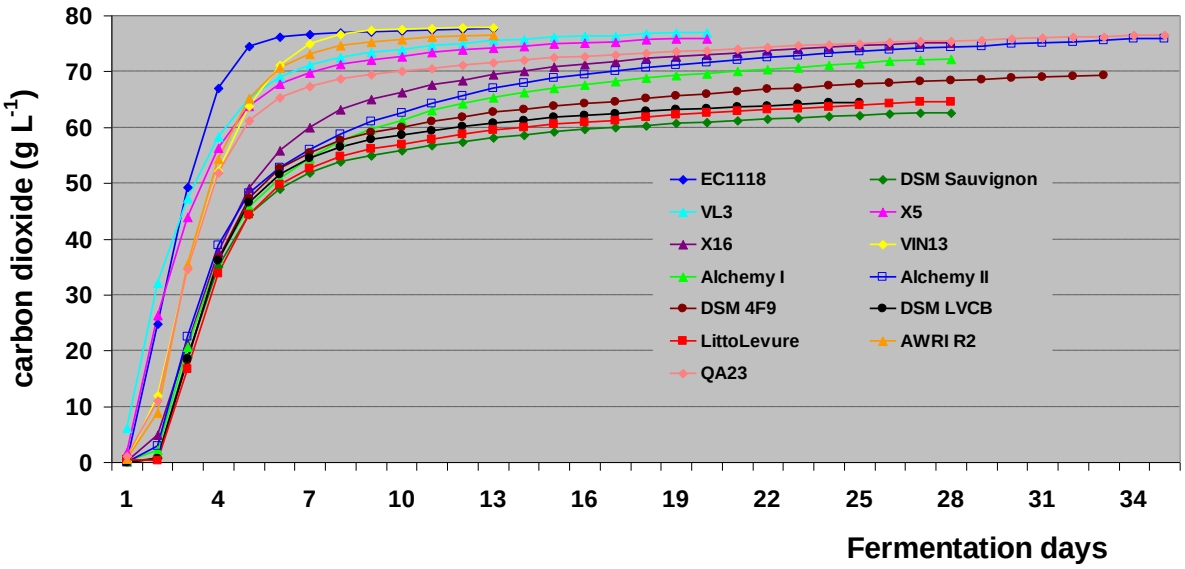


Figure 4-8 Growth kinetics of fresh Scheurebe grape juice fermentations with 13 different commercial yeast strains

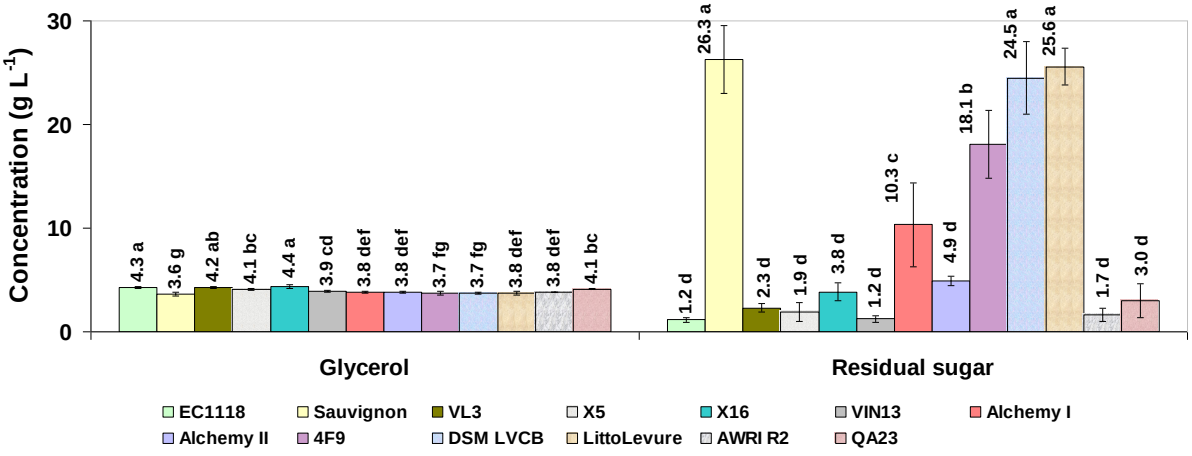


Figure 4-9 Concentration of residual sugar and glycerol in finished Scheurebe grape wines produced by 13 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each compound according to the DMRT test.

Table 4-5 Concentration of organic acids found in finished Scheurebe wines developed by 13 yeast strains

Yeast	Tartaric acid (g L^{-1})	Malic acid (g L^{-1})	Lactic acid (g L^{-1})	Shikimic acid (mg L^{-1})	Acetic acid (g L^{-1})	Citric acid (g L^{-1})
EC1118	0.90 ± 0.09 a	5.18 ± 0.46 a	0.14 ± 0.00 a	43.27 ± 1.00 a	0.24 ± 0.01 b	0.22 ± 0.02 abc
Sauvignon	1.00± 0.15 a	4.99 ± 0.10 a	0.08 ± 0.02 def	43.57 ± 0.42 a	0.39 ± 0.02 a	0.19 ± 0.01 cd
VL3	1.00 ± 0.18 a	5.01 ± 0.06 a	0.08 ± 0.02 def	44.52 ± 0.77 a	0.37 ± 0.01 a	0.25 ± 0.02 a
X5	0.85 ± 0.08 a	4.99 ± 0.09 a	0.09 ± 0.01 cde	42.79 ± 0.40 a	0.36 ± 0.03 a	0.21 ± 0.01 bc
X16	1.01 ± 0.12 a	4.90 ± 0.10 a	0.06 ± 0.01 f	43.88 ± 0.93 a	0.38 ± 0.02 a	0.22 ± 0.01 abc
VIN13	0.91 ± 0.08 a	5.02 ± 0.11 a	0.10 ± 0.01 bcd	43.91 ± 0.62 a	0.20 ± 0.02 b	0.18 ± 0.01 d
Alchemy I	1.02 ± 0.18 a	5.04 ± 0.19 a	0.07 ± 0.01 ef	43.80 ± 0.17 a	0.34 ± 0.01 a	0.18 ± 0.00 d
Alchemy II	1.23 ± 0.30 a	4.83 ± 0.16 a	0.08 ± 0.01 def	43.83 ± 1.00 a	0.37 ± 0.02 a	0.19 ± 0.01 cd
4F9	1.20 ± 0.24 a	5.03 ± 0.21 a	0.08 ± 0.01 def	44.00 ± 2.25 a	0.36 ± 0.04 a	0.19 ± 0.00 cd
LVCB	0.93 ± 0.10 a	5.23 ± 0.01 a	0.08 ± 0.01 def	43.47 ± 0.30 a	0.37 ± 0.04 a	0.19 ± 0.00 cd
LittoLevure	1.06 ± 0.26 a	5.13 ± 0.13 a	0.08 ± 0.01 def	43.54 ± 0.32 a	0.38 ± 0.01 a	0.19 ± 0.00 cd
AWRI R2	0.88 ± 0.07 a	5.00 ± 0.18 a	0.12 ± 0.00 b	44.23 ± 0.13 a	0.34 ± 0.03 a	0.19 ± 0.00 cd
QA23	1.08 ± 0.32 a	5.21 ± 0.09 a	0.10 ± 0.02 bc	45.19 ± 1.38 a	0.34 ± 0.06 a	0.25 ± 0.06 a

Each value shows the mean ± standard deviation.

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among yeast strains, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

SO_2-binding compounds: As shown in **Figure 4-10**, strains VL3, X5 and X16 were the highest producers of not only α-ketoglutarate (32.4 ± 1.8, 32.8 ± 1.4 and 26.3 ± 1.3 mg L^{-1}, respectively), but also acetaldehyde (62.7 ± 0.4, 65.1 ± 3.7 and 62.0 ± 7.7 mg L^{-1}, respectively), however produced low pyruvate. Strain EC1118 was the highest producer of α-ketoglutarate and pyruvate (27.7 ± 1.8 and 29.0 ± 3.4 mg L^{-1}), but the lowest producer of acetaldehyde (41.9 ± 1.1 mg L^{-1}). In addition, acetaldehyde concentration was also most evident in the Sauvignon, Alchemy I, 4F9, LVCB and LittoLevure wines (63.1 ± 1.4, 55.7 ± 2.7, 56.6 ± 6.4, 58.5 ± 1.6 and 64.4 ± 4.0 mg L^{-1}, respectively). Interestingly, strain VIN13 seemed to be the lowest SO_2-binding compound producer.

Sulphur containing compounds: **Figure 4-11** displays some differences among the yeast strains in terms of H_2S, dimethyl sulphide (DMS) and carbon disulphide (CS_2) in the Scheurebe wines. The Sauvignon strain was the highest producer of H_2S and CS_2 (3.4 ± 0.6 and 2.3 ± 0.2 μg L^{-1}, respectively) followed by strains EC1118, X16 and VIN13, respectively. The concentration of CS_2 was also most evident in the QA23 wine (2.1 ± 0.1 μg L^{-1}), but H_2S was not detectable in this wine. The thirteen yeast strains developed similar amounts of DMS ranging from 1.1 ± 0.1 to 1.7 ± 0.2 μg L^{-1}). The other investigated sulphur compounds, ethanethiol, methanethiol, dimethyl disulphide, thioacetic acid S-methylester and thioacetic acid S-ethylester were not detected in all wine treatments.

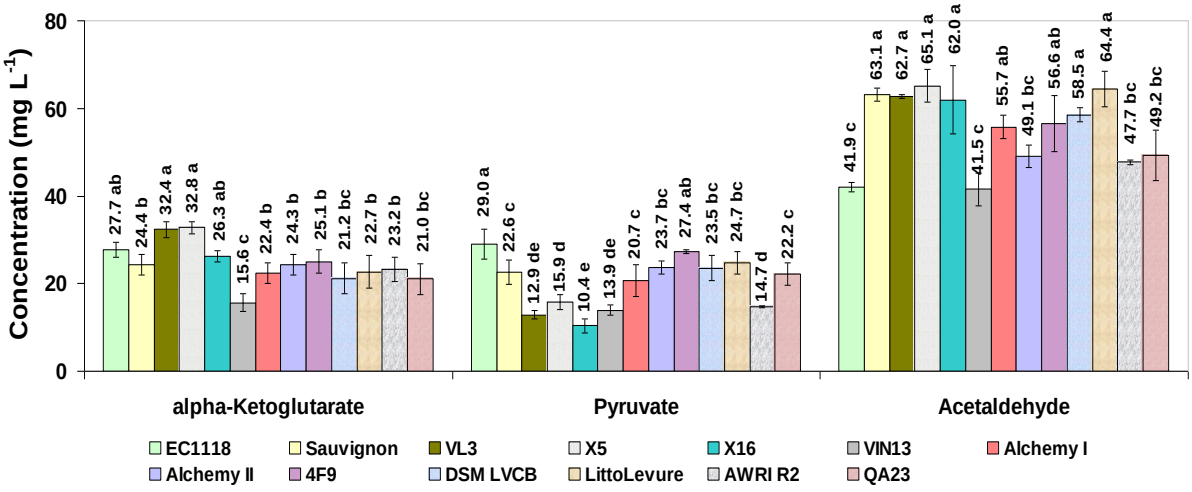


Figure 4-10 Concentration of SO_2-binding compounds in finished Scheurebe grape wines produced by 13 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among the yeast strains of each compound according to the DMRT test.

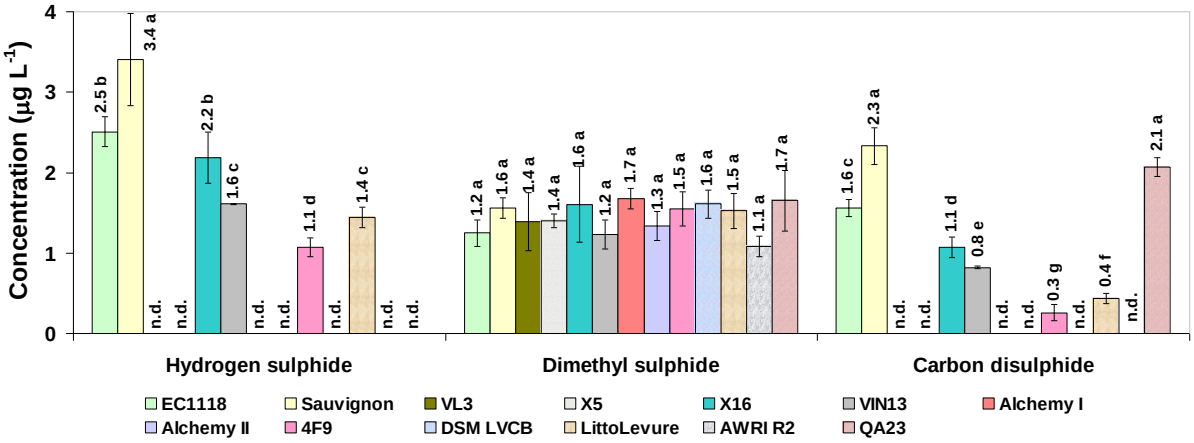


Figure 4-11 Formation of hydrogen sulphide, dimethyl sulphide and carbon disulphide in finished Scheurebe grape wines produced by 13 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each compound, whereas means followed by the same letters are not significantly different ($p>0.05$) according to the DMRT test.

Higher alcohols: As shown in **Table 4-6**, the production of 2-methyl propanol and 3-methyl butanol slightly differed among the yeast strains used. Strain EC1118 seemed to be the predominant producer of the last two alcohols (28.7 $\pm$ 3.5 and 153.3 $\pm$ 16.8 mg L^{-1}, respectively). The highest concentration of 2-methyl propanol was also produced by strain Alchemy II (29.3 $\pm$ 6.1 mg L^{-1}), followed by strains X16, 4F9, LVCB and AWRI 2 (24.7 $\pm$ 0.6, 25.0 $\pm$ 6.2, 26.7 $\pm$ 10.4 and 26.3 $\pm$ 2.5 mg L^{-1}, respectively). The formation of hexanol and 2-methyl butanol behaved similarly across the thirteen yeast strains used. They produced similar concentrations ranging from 1745.7 $\pm$ 198.8 to 2187.3 $\pm$ 126.5 mg L^{-1} of hexanol and 18.0 $\pm$ 3.0 to 26.0 $\pm$ 4.6 mg L^{-1} of 2-methyl butanol. Regarding the 2-phenyl ethanol, its concentration was most evident in the VL3 wine (24.4 $\pm$ 1.7 mg L^{-1}) followed by the QA23, EC1118 and X16 wines (23.8 $\pm$ 0.5, 23.3 $\pm$ 1.6, 23.3 $\pm$ 1.6 and 23.4 $\pm$ 0.7 mg L^{-1}, respectively) as shown in **Figure 4-12**.

Acetic acid esters: The results in **Figure 4-12** and **Figure 4-13** demonstrate significantly different formation of acetic acid esters that varied among the commercial yeast strains used. The VIN13 strain was the most predominant acetic acid ester producer, except for acetic acid ethyl ester. It produced wine containing the highest amounts of acetic acid 3-methylbytyl ester, acetic acid 2-methylbytyl ester, acetic acid 2-phenylethyl ester and acetic acid hexyl ester ranging from 800.6 $\pm$ 128.2 µg L^{-1}, 49.1 $\pm$ 4.3 µg L^{-1}, 124.1 $\pm$ 1.5 µg L^{-1} and 136.3 $\pm$ 2.2 µg L^{-1}. Interestingly, strains 4F9 and LittoLevure developed only trace amounts of

acetic acid ethyl ester, while the remaining yeast strains (excluding VIN13) produced similar high amounts ranging from 53.3 ± 5.1 to 90.0 ± 12.0 mg L^{-1} (**Figure 4-12**). Strain EC1118 also seemed to produce high amounts of most acetic acid esters, although slightly lower than the formation by strain VIN13. High concentrations of acetic acid 2-phenylethyl ester were also evident in the AWRI R2 and VL3 wines (107.4 ± 7.2 and 104.1 ± 3.4 μg L^{-1}, respectively). It seemed that there was a positive correlation between the higher concentration of 2-phenyl ethanol (**Figure 4-12**) and the greater acetic acid 2-phenyl ethyl ester formation by strains EC1118 and VL3 (**Figure 4-13**).

Table 4-6 Concentration of higher alcohols found in finished Scheurebe wines developed by 13 yeast strains

Yeast	2-Methyl propanol (mg L^{-1})	Hexanol (μg L^{-1})	3-Methyl butanol (mg L^{-1})	2-Methyl butanol (mg L^{-1})
EC1118	28.7 ± 3.5 a	2187.3 ± 126.5 a	153.3 ± 16.8 a	25.3 ± 3.1 a
Sauvignon	22.0 ± 3.0 abc	1909.0 ± 105.0 a	96.3 ± 6.1 bc	22.0 ± 1.7 a
VL3	20.7 ± 2.3 abc	2033.7 ± 108.2 a	113.3 ± 11.5 bc	22.7 ± 2.3 a
X5	14.7 ± 2.5 c	1885.0 ± 115.4 a	94.0 ± 15.0 bc	18.0 ± 3.0 a
X16	24.7 ± 0.6 ab	2147.0 ± 91.8 a	108.7 ± 1.5 bc	25.3 ± 0.6 a
VIN13	17.0 ± 3.6 bc	1758.0 ± 198.6 a	104.0 ± 18.5 bc	21.0 ± 3.0 a
Alchemy I	21.7 ± 1.5 abc	2031.0 ± 45.9 a	105.7 ± 4.2 bc	21.3 ± 1.1 a
Alchemy II	29.3 ± 6.1 a	2039.3 ± 167.3 a	119.7 ± 17.6 bc	26.0 ± 4.6 a
4F9	25.0 ± 6.2 ab	1745.7 ± 198.8 a	96.7 ± 18.1 bc	22.3 ± 4.7 a
LVCB	26.7 ± 10.4 ab	1863.3 ± 306.2 a	102.7 ± 32.8 bc	23.7 ± 8.4 a
LittoLevure	20.7 ± 8.0 abc	1694.0 ± 302.1 a	85.7 ± 32.2 c	19.7 ± 8.0 a
AWRI R2	26.3 ± 2.5 ab	2088.7 ± 113.8 a	118.7 ± 11.9 bc	24.7 ± 3.1 a
QA23	20.3 ± 4.9 abc	1991.0 ± 447.5 a	127.0 ± 15.1 ab	22.0 ± 4.6 a

Each value shows the mean ± standard deviation.

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among the yeast strains, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

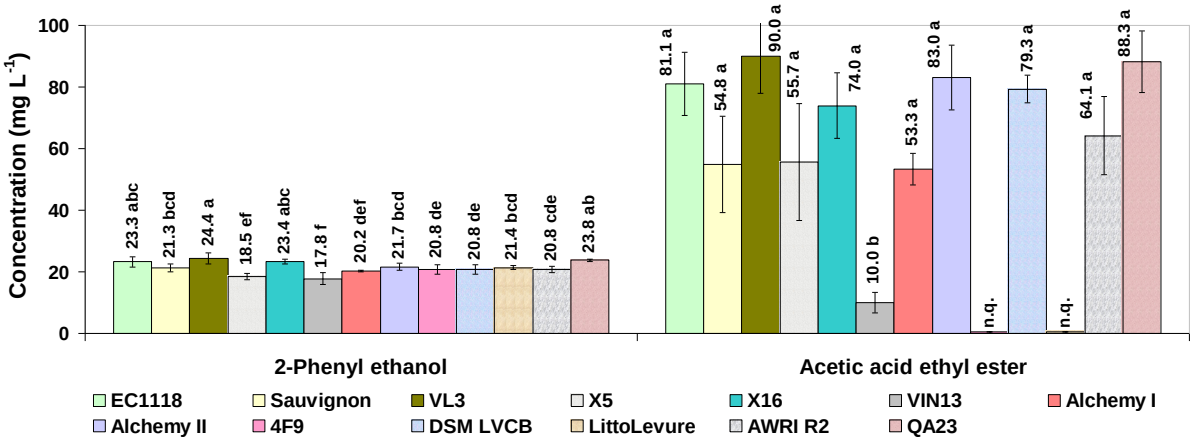


Figure 4-12 Formation of 2-phenyl ethanol and acetic acid ethyl ester in finished Scheurebe grape wines produced by 13 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among the yeast strains of each compound according to the DMRT test.

n.q. denotes not quantified or traced

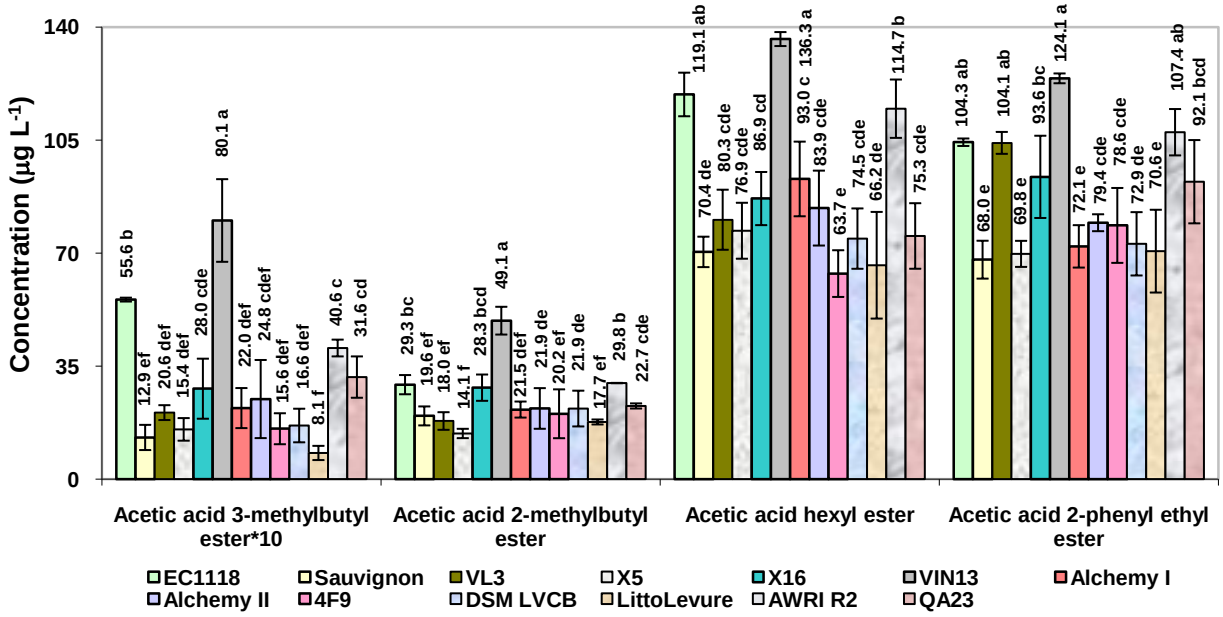


Figure 4-13 Formation of acetic acid esters found in finished Scheurebe grape wines developed by 13 different yeast strains (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each compound according to the DMRT test.

Table 4-7 Concentration of ethyl esters and diethyl ester found in finished Scheurebe wines fermented with 13 yeast strains

Yeast	PrEtE (μg L^{-1})	BuEtE (μg L^{-1})	iBuEtE (μg L^{-1})	LAEtE (mg L^{-1})	SAdiEtE (μg L^{-1})
EC1118	119.3 ± 10.2 a	215.0 ± 11.4 abc	58.3 ± 5.1 bcd	25.7 ± 1.5 a	1467.3 ± 88.2 a
Sauvignon	30.0 ± 2.7 cd	263.0 ± 17.8 ab	42.7 ± 4.2 efg	13.7 ± 0.6 de	334.3 ± 68.1 d
VL3	51.3 ± 7.0 bc	143.0 ± 10.5 de	74.7 ± 7.1 a	15.0 ± 1.0 cde	1577.7 ± 140.0 a
X5	64.7 ± 15.5 b	121.7 ± 14.0 e	38.7 ± 6.5 fg	15.3 ± 0.6 cde	1562.0 ± 20.3 a
X16	48.7 ± 2.5 bc	240.3 ± 17.0 abc	70.0 ± 3.0 ab	14.0 ± 0.0 de	1171.7 ± 107.9 b
VIN13	53.3 ± 5.5 bc	221.7 ± 19.6 abc	33.0 ± 3.6 g	16.0 ± 2.0 cd	1238.0 ± 11.8 b
Alchemy I	34.3 ± 2.1 cd	229.7 ± 9.0 abc	44.7 ± 0.6 defg	14.3 ± 0.6 cde	661.0 ± 83.4 c
Alchemy II	39.7 ± 8.5 cd	248.0 ± 32.1 abc	56.3 ± 7.1 cde	15.7 ± 1.5 cde	714.0 ± 26.5 c
4F9	30.3 ± 10.2 cd	259.7 ± 53.0 ab	46.3 ± 11.6 defg	13.7 ± 2.1 de	454.3 ± 208.8 d
LVCB	31.0 ± 14.8 cd	273.3 ± 55.7 a	45.0 ± 12.5 defg	14.0 ± 2.6 de	377.3 ± 121.1 d
LittoLevure	22.0 ± 2.7 d	225.3 ± 51.8 abc	35.0 ± 12.1 fg	13.0 ± 2.0 e	405.7 ± 29.5 d
AWRI R2	52.0 ± 6.0 bc	188.0 ± 17.1 cd	67.7 ± 5.5 abc	18.7 ± 0.6 b	740.7 ± 57.9 c
QA23	67.3 ± 15.6 b	210.0 ± 46.5 bc	48.3 ± 6.8 def	17.0 ± 1.0 bc	805.0 ± 124.9 c

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying different letters within the same column are significantly different ($p<0.05$) according to the DMRT test.

Ethyl esters and diethyl ester: The results in **Table 4-7** show the difference of these compounds among the thirteen yeast strains inoculated. The EC1118 strain was the most predominant strain of PrEtE, LAEtE and SAdiEtE productions (119.3 ± 10.2 μg L^{-1}, 25.7 ± 1.5 mg L^{-1} and 1467.3 ± 88.2 μg L^{-1}, respectively). Concentration of SAdiEtE was also most obvious in the VL3 and X5 wines (1577.7 ± 140.0 and 1562.0 ± 20.3 μg L^{-1}, respectively). Strain LVCB developed the highest amounts of BuEtE (273.3 ± 55.7 μg L^{-1}) followed by strains Sauvignon and 4F9 (263.0 ± 17.8 and 259.7 ± 53.0 μg L^{-1}, respectively), while low amounts were produced by the VL3 and X5 strains. The highest concentration of iBuEtE was present in the VL3 and X16 wines (74.7 ± 7.1 and 70.0 ± 3.0 μg L^{-1}, respectively) followed by the AWRI R2 wine (67.7 ± 5.5 μg L^{-1}).

Medium-chain fatty acids and their esters: Like the other aroma compounds, concentrations of MCFAs and their ethyl esters were significantly influenced by the commercial yeast strains inoculated, except for DAEtE as given in **Table 4-8**. The thirteen yeast strains produced similar concentrations of DAEtE ranging from 403.7 ± 158.2 to 565.0 ± 13.2 μg L^{-1}. The five strains of Alchemy I and II, 4F9, LVCB and LittoLevure similarly developed the highest amounts of not only hexanoic, octanoic and decanoic acids (12.3 ± 0.6 to 14.0 ± 1.7 mg L^{-1}, 11.0 ± 0.0 to 13.0 ± 1.0 mg L^{-1} and 2.6 ± 0.1 to 2.9 ± 0.3 mg L^{-1}, respectively) but also HAEtE and OAEtE ranging from 949.7 ± 72.2 to 988.7 ± 68.6 μg L^{-1} and 1426.7 ± 104.9 to 1590.0 ± 58.8 μg L^{-1}, respectively. Strain Sauvignon also produced high amounts of these compounds, except for decanoic acid. High amounts of decanoic acid were also present in the VIN13 wine. On the contrary, the VL3 strain tended to be the low producer of these compounds, excluding DAEtE.

Monoterpenes: **Figure 4-14** demonstrates that the thirteen yeast strains significantly affected only the liberation of linalool. The X5, VIN13, Alchemy II and VL3 strains similarly released high amounts of linalool (42.6 ± 1.7, 42.9 ± 2.2, 42.6 ± 2.1 and 41.3 ± 0.7 μg L^{-1}, respectively), on the contrary, strains EC1118 and Sauvignon seemed to liberate low amounts (36.8 ± 1.5 and 36.3 ± 1.0 μg L^{-1}, respectively). Other monoterpenes, *trans*-linalool oxide, *cis*-linalool oxide and α-terpineol were liberated similarly among all yeast strains that ranged from 127.0 ± 5.5 to 136.8 ± 5.9 μg L^{-1}, 31.8 ± 2.4 to 34.3 ± 0.6 μg L^{-1} and 70.4 ± 8.7 to 79.3 ± 6.5 μg L^{-1}, respectively.

Table 4-8 Concentration of fatty acids and their ethyl esters found in finished Scheurebe wines fermented with 13 yeast strains

Yeast	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})	HAEtE (μg L^{-1})	OAEtE (μg L^{-1})	DAEtE (μg L^{-1})
EC1118	9.0 ± 0.0 b	9.3 ± 0.6 cd	2.0 ± 0.1 abc	698.0 ± 10.6 de	1343.0 ± 32.0 ab	531.0 ± 15.9 a
Sauvignon	12.7 ± 1.5 a	11.0 ± 2.0 abc	1.6 ± 1.1 c	960.3 ± 46.3 abc	1425.7 ± 78.2 a	447.3 ± 43.5 a
VL3	7.0 ± 0.0 c	7.0 ± 0.0 d	1.6 ± 0.1 c	488.7 ± 26.5 f	1011.7 ± 47.1 b	427.0 ± 7.2 a
X5	7.0 ± 0.0 c	7.3 ± 0.6 d	2.0 ± 0.1 abc	482.7 ± 37.5 f	1003.3 ± 88.9 b	461.3 ± 8.3 a
X16	10.0 ± 1.0 b	8.7 ± 1.5 cd	1.9 ± 0.6 bc	827.3 ± 61.0 bcd	1215.3 ± 248.5 ab	403.7 ± 158.2 a
VIN13	9.7 ± 0.6 b	10.0 ± 0.0 bc	2.5 ± 0.1 ab	809.0 ± 47.3 cde	1443.3 ± 59.5 a	565.0 ± 13.2 a
Alchemy I	12.3 ± 0.6 a	11.0 ± 0.0 abc	2.6 ± 0.1 ab	973.0 ± 41.6 ab	1440.3 ± 137.1 a	432.7 ± 50.9 a
Alchemy II	13.0 ± 0.0 a	12.0 ± 0.0 ab	2.7 ± 0.2 ab	988.7 ± 68.6 a	1590.0 ± 58.8 a	513.7 ± 13.6 a
4F9	13.3 ± 0.6 a	12.7 ± 0.6 a	2.9 ± 0.3 a	949.7 ± 72.2 abc	1433.7 ± 262.5 a	513.7 ± 84.7 a
LVCB	13.0 ± 1.0 a	12.3 ± 0.6 ab	2.7 ± 0.3 ab	954.7 ± 62.0 abc	1448.3 ± 153.0 a	430.7 ± 39.0 a
LittoLevure	14.0 ± 1.7 a	13.0 ± 1.0 a	2.8 ± 0.5 ab	982.7 ± 24.9 ab	1426.7 ± 104.9 a	479.7 ± 141.4 a
AWRI R2	8.3 ± 0.6 bc	9.0 ± 0.0 cd	2.0 ± 0.1 abc	659.0 ± 59.0 e	1260.7 ± 9.0 ab	504.7 ± 31.7 a
QA23	10.0 ± 2.6 b	9.0 ± 3.5 cd	1.9 ± 0.9 bc	768.7 ± 46.3 de	1276.7 ± 135.7 ab	407.0 ± 104.5 a

Each value shows the mean ± standard deviation.

Values displaying the same letter (only ‘a’) within the same column of each factor indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

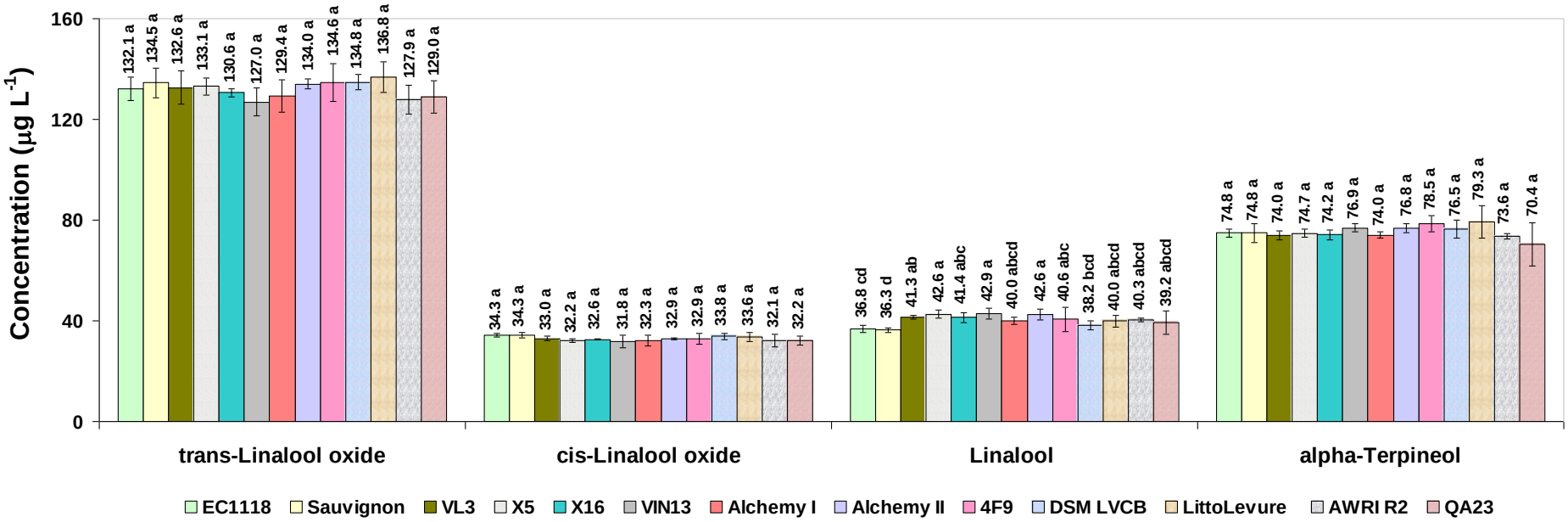


Figure 4-14 Formation of monoterpenes found in finished Scheurebe grape wines developed by 13 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each monoterpene, whereas means followed by the same letters are not significantly different ($p>0.05$) according to the DMRT test.

4.2 Effects of yeast strains and/or nutrient supplements on wine fermentation and quality of Sauvignon blanc grape wines

The experimental results which are described in the following chapters were obtained from Sauvignon blanc grape juice fermented with different commercial *Saccharomyces* yeast strains and/or nutrient sources at different concentrations.

4.2.1 Effect of nine commercial *Saccharomyces* yeast strains and two nutrient sources on fermentative characteristics, metabolic compounds and wine aromas of Sauvignon blanc grape wines

In 2008, fermentation was performed in fresh Sauvignon blanc juices. Nine commercial yeast strains; EC1118, Sauvignon, VL3, X5, VIN7, VIN13, 4F9, LVCB and AWRI R2 and two nutrient sources; OptiWhite (natural inactivated yeast) and Fermaid E (blend of DAP, thiamine, yeast cell walls and ammonium sulphate) at 0.3 g L^{-1} were used in the fermentations. The fermentation kinetics, some metabolic compounds and wine aromas in final wines were examined.

The fermentation kinetics: The result shows that all fermentation kinetics had a similar pattern among nutrient sources but varied depending on the nine yeast strains used (**Figure 4-15**). Strains AWRI R2, X5, VIN13 and VL3 seemed to be the strongest strains and had similarly faster growth kinetics and shorter fermentation times (11 days). While strains EC1118, LVCB and 4F9 followed similar patterns, they had however a slightly longer lag phase. On the other hand, the Sauvignon and VIN7 strains had slow growth kinetics and fermentation times (16 days), particularly the latter strain seemed to obtain a sluggish fermentation with four days of lag phase after inoculation. Nevertheless, all treatments completely finished fermentation depending on the yeast strains. Overall, the addition of Fermaid E tended to enhance more effective the fermentation kinetic than OptiWhite.

Chemical composition: Both yeast strain and nutrient source had no influence on the concentrations of residual sugar and glycerol in final wines (**Figure 4-16**). The nine yeast strains produced wines having similar amounts of residual sugar and glycerol ranging from 0.3 $\pm$ 0.1 to 0.9 $\pm$ 0.2 g L^{-1} and 4.5 $\pm$ 0.0 to 6.3 $\pm$ 0.1 g L^{-1}, respectively. All yeast strains consumed practically almost initial sugar provided and consequently resulted in low amounts of residual sugar below 1 g L^{-1}. Nonetheless, strains EC1118, VL3, VIN7 and VIN13 tended to produce high amounts of glycerol.

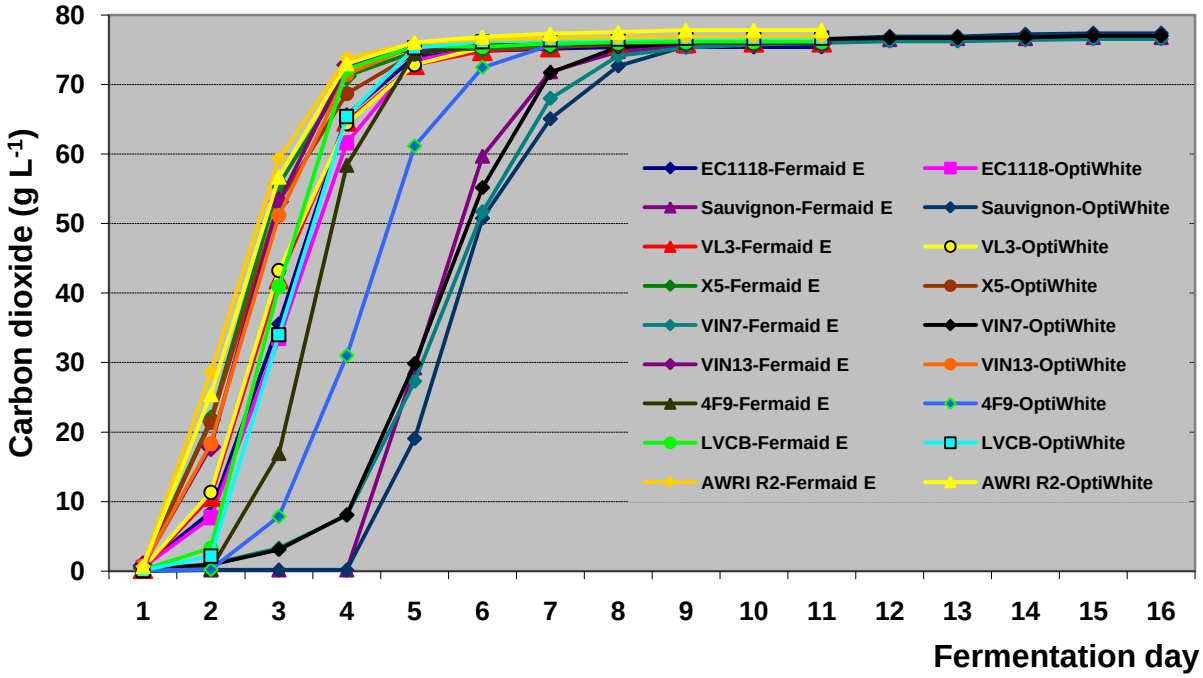


Figure 4-15 Fermentation kinetics of fresh Sauvignon blanc grape juice fermentations with nine different commercial yeast strains and two nutrient sources

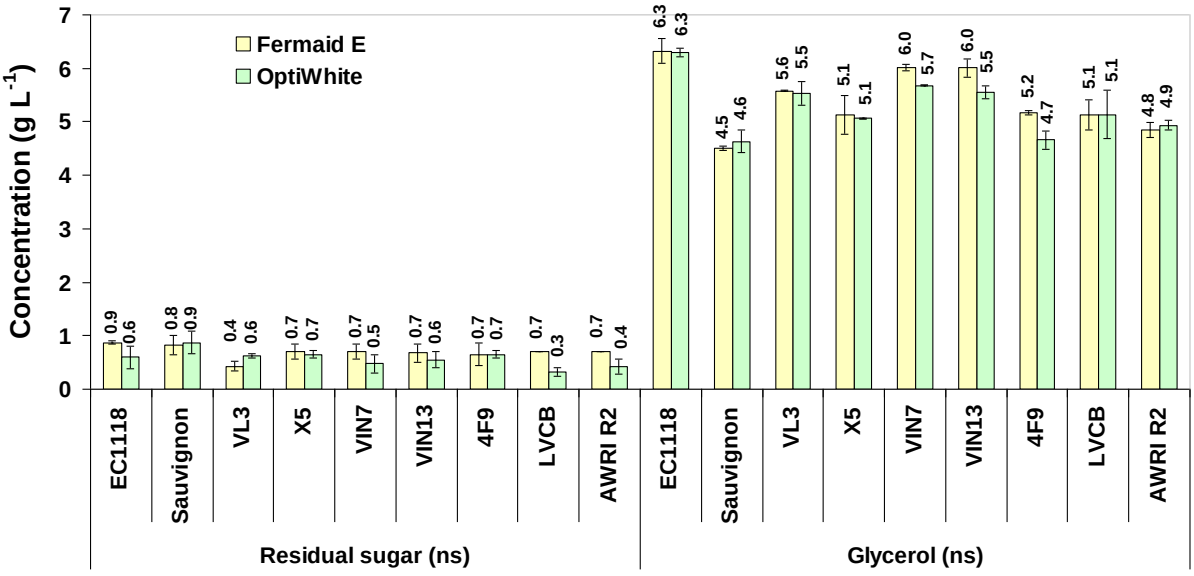


Figure 4-16 Concentration of residual sugar and glycerol found in finished Sauvignon blanc grape wines fermented with nine yeast strains and two nutrient sources

Vertical bars represent standard deviations from two fermentation replicates. Compounds displaying **"(ns)"** show no significant difference ($p>0.05$) among treatment combinations of each compound according to the DMRT test.

Table 4-9 Concentration of organic acids found in finished Sauvignon blanc wines produced by nine yeast strains with two nutrient sources

Yeast	Nutrient	Tartaric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Lactic acid ($g\ L^{-1}$)	Shikimic acid ($mg\ L^{-1}$)	Acetic acid ($g\ L^{-1}$)	Citric acid ($g\ L^{-1}$)
EC1118	Fermaid E	3.34 ± 0.08 a	5.29 ± 0.05 bc	0.19 ± 0.00 b	34.03 ± 0.19 a	0.29 ± 0.00 cde	0.27 ± 0.00 b
	OptiWhite	3.19 ± 0.02 a	5.35 ± 0.01 ab	0.16 ± 0.00 ef	33.06 ± 0.07 a	0.30 ± 0.00 cde	0.27 ± 0.00 b
Sauvignon	Fermaid E	3.14 ± 0.16 a	5.16 ± 0.05 de	0.16 ± 0.00 ef	34.77 ± 0.17 a	0.30 ± 0.02 cde	0.32 ± 0.00 a
	OptiWhite	3.04 ± 0.16 a	5.08 ± 0.02 ef	0.12 ± 0.00 ij	34.18 ± 0.69 a	0.40 ± 0.00 b	0.28 ± 0.00 b
VL3	Fermaid E	3.38 ± 0.11 a	5.00 ± 0.08 fg	0.13 ± 0.01 hi	33.80 ± 0.63 a	0.29 ± 0.00 cde	0.25 ± 0.00 b
	OptiWhite	3.38 ± 0.13 a	4.97 ± 0.03 g	0.11 ± 0.02 j	33.89 ± 0.31 a	0.31 ± 0.03 cd	0.26 ± 0.00 b
X5	Fermaid E	3.23 ± 0.07 a	5.17 ± 0.01 de	0.19 ± 0.00 b	34.06 ± 0.72 a	0.23 ± 0.03 ef	0.25 ± 0.00 b
	OptiWhite	3.26 ± 0.25 a	5.20 ± 0.01 cd	0.15 ± 0.00 fg	34.24 ± 0.00 a	0.32 ± 0.01 cd	0.26 ± 0.00 b
VIN7	Fermaid E	3.54 ± 0.21 a	5.19 ± 0.02 cd	0.17 ± 0.01 cde	33.11 ± 0.33 a	0.65 ± 0.05 a	0.26 ± 0.00 b
	OptiWhite	2.89 ± 0.42 a	5.20 ± 0.06 cd	0.18 ± 0.01 cd	32.66 ± 0.15 a	0.63 ± 0.01 a	0.26 ± 0.00 b
VIN13	Fermaid E	3.50 ± 0.07 a	5.16 ± 0.03 de	0.17 ± 0.01 de	33.68 ± 0.05 a	0.34 ± 0.01 bc	0.27 ± 0.00 b
	OptiWhite	3.25 ± 0.34 a	5.15 ± 0.04 de	0.12 ± 0.02 ij	33.43 ± 0.35 a	0.29 ± 0.01 cde	0.28 ± 0.00 b
4F9	Fermaid E	3.03 ± 0.28 a	5.44 ± 0.03 a	0.22 ± 0.02 a	33.45 ± 0.05 a	0.24 ± 0.07 ef	0.26 ± 0.00 b
	OptiWhite	3.26 ± 0.33 a	5.44 ± 0.07 a	0.16 ± 0.01 ef	33.15 ± 0.13 a	0.30 ± 0.02 cde	0.27 ± 0.00 b
LVCB	Fermaid E	2.87 ± 0.35 a	5.20 ± 0.01 cd	0.22 ± 0.01 a	34.12 ± 0.10 a	0.19 ± 0.07 f	0.26 ± 0.00 b
	OptiWhite	3.04 ± 0.04 a	5.41 ± 0.00 a	0.17 ± 0.01 de	33.25 ± 0.14 a	0.25 ± 0.01 def	0.26 ± 0.00 b
AWRI R2	Fermaid E	3.16 ± 0.10 a	5.36 ± 0.08 ab	0.19 ± 0.01 b	34.16 ± 0.26 a	0.26 ± 0.02 def	0.26 ± 0.00 b
	OptiWhite	3.08 ± 0.18 a	5.43 ± 0.01 a	0.14 ± 0.00 gh	34.25 ± 0.97 a	0.29 ± 0.09 cde	0.27 ± 0.00 b

Each value shows the mean ± standard deviation. Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$) among treatments, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Organic acids: The results in **Table 4-9** demonstrate that strains 4F9 and AWRI R2 produced wines containing the highest amounts of malic acid with less response to nutrient source (5.44 ± 0.03, 5.44 ± 0.07 and 5.36 ± 0.08, 5.43 ± 0.01 g L^{-1}, respectively). Whereas, the presence of OptiWhite resulted in high amounts of this acid in the LVCB and EC1118 wines (5.41 ± 0.00 and 5.35 ± 0.01 g L^{-1}). The results also showed that strains 4F9 and AWRI R2 consumed approximately 22.5 % of malic acid from the initial acid (7.02 g L^{-1}). On the other hand, addition of Fermaid E to the juice resulted in the highest level of lactic acid in the 4F9 and LVCB wines (0.22 ± 0.02 and 0.22 ± 0.01 g L^{-1}) and citric acid in the Sauvignon wine (0.32 ± 0.00 g L^{-1}). Strain VIN7 was the highest acetic acid producer in either the Fermaid E or the OptiWhite treatment (0.65 ± 0.05 and 0.63 ± 0.01 g L^{-1}, respectively). All wine treatments had similar concentrations of tartaric and shikimic acid ranging from 2.87 ± 0.35 to 3.54 ± 0.21 g L^{-1} and 32.66 ± 0.15 to 34.77 ± 0.17 mg L^{-1}, respectively.

SO_2-binding compounds: The results show that different productions of α-ketoglutarate and pyruvate in wines significantly varied depending on the yeast strains and the nutrient sources, while that of acetaldehyde were similar (**Figure 4-17**). The addition of OptiWhite into the juice significantly stimulated the highest formation of α-ketoglutarate by strains Sauvignon and X5 (45.8 ± 2.3 and 43.1 ± 0.3 mg L^{-1}) and that of pyruvate by the VIN7 strain (28.8 ± 4.3 mg L^{-1}). Nevertheless, the concentration of these keto acids significantly diminished with Fermaid E supplementation. Regarding acetaldehyde, its concentrations were similar among the wine treatments ranging from 9.1 ± 2.4 to 27.2 ± 0.0 mg L^{-1}, although strain EC1118 tended to be a high acetaldehyde producer.

Sulphur containing compounds: **Figure 4-18** demonstrates that the nine yeast strains exhibited different production of these compounds in response to the nutrient source. Strain VIN7 constantly produced the highest amounts of dimethyl sulphide in both nutrient sources (10.3 ± 1.0 and 10.6 ± 0.2 μg L^{-1}, respectively). Carbon disulphide was detected only in the Sauvignon, X5, VIN7, VIN13 and LVCB wines, however was most evident in the LVCB wine (1.3 ± 0.2 and 1.1 ± 0.0 μg L^{-1}, respectively). Interestingly, strain LVCB also developed the highest amount of dimethyl disulphide in the Fermaid E sample (1.0 ± 0.3 μg L^{-1}). In addition, other sulphur containing compounds like hydrogen sulphide, ethanethiol, methanethiol, thioacetic acid S-methylester and thioacetic acid S-ethylester were not detected in all Sauvignon blanc wines.

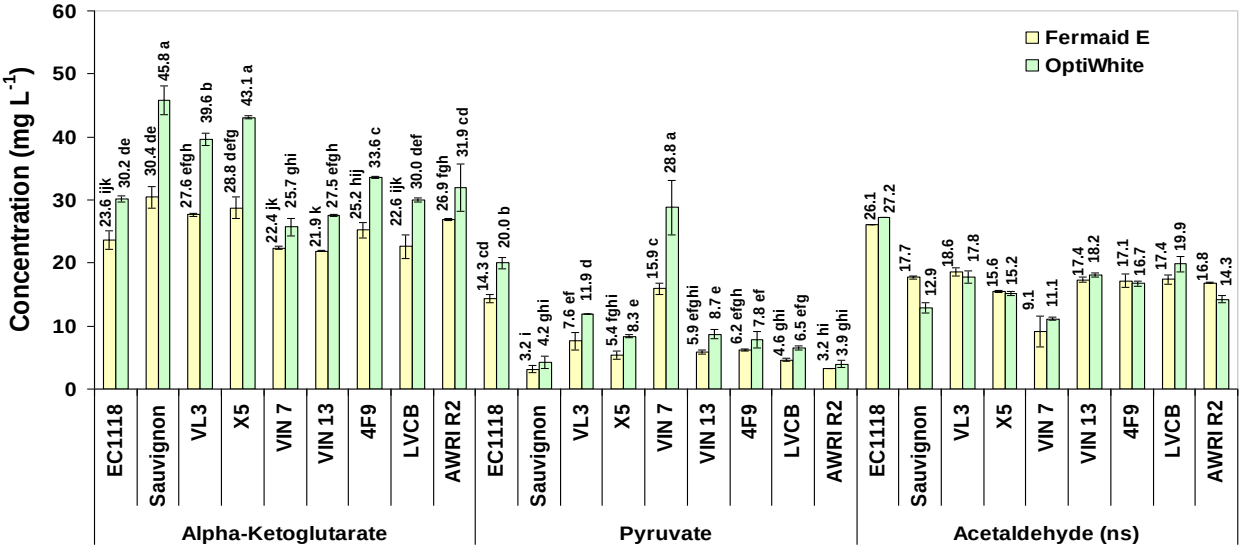


Figure 4-17 Concentration of SO_2-binding compounds in finished Sauvignon blanc grape wines fermented with nine yeast strains and two nutrient sources

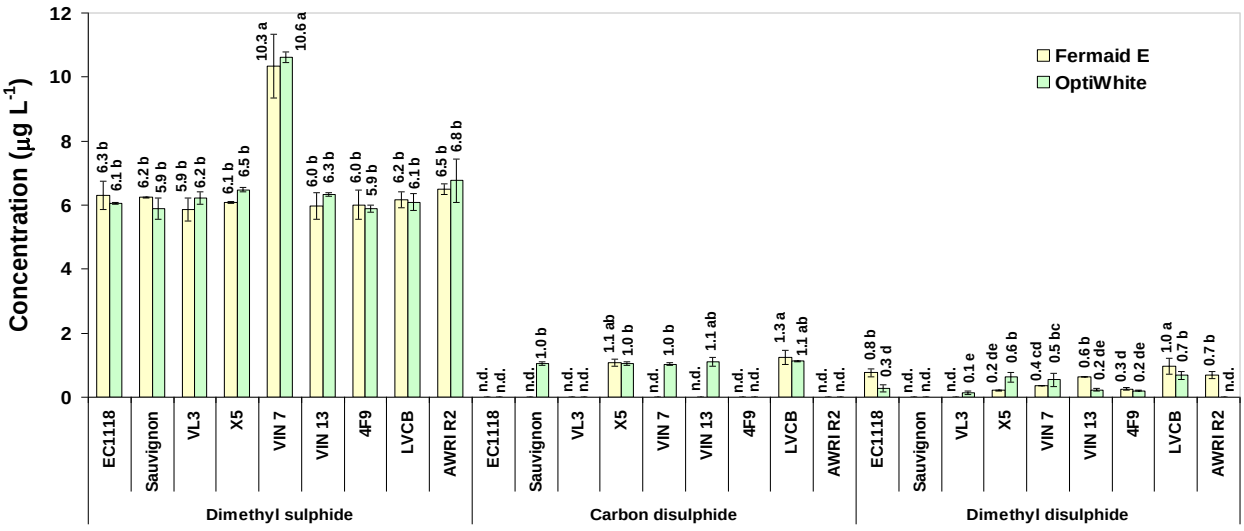


Figure 4-18 Concentration of dimethyl sulphide, carbon disulphide and dimethyl disulphide found in finished Sauvignon blanc grape wines produced by nine yeast strains and two nutrient sources

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test. Compounds displaying **"(ns)"** show no significant difference ($p>0.05$) among treatment combinations of each compound according to the DMRT test.

n.d. denotes not detected

Higher alcohols: The results in **Table 4-10** show that the interaction of the commercial yeast strain and the nutrient source did not significantly influence the formation of all five higher alcohols in final wines. However, the AWRI R2 and Sauvignon strains considerably tended to be higher alcohol producers and the addition of OptiWhite tended to stimulate the higher development of these alcohols for the nine yeast strains. The Sauvignon blanc wines had similar concentrations of higher alcohols; 17.0 $\pm$ 1.5 to 47.5 $\pm$ 6.7 mg L^{-1} 2-methyl propanol, 103.8 $\pm$ 14.1 to 176.3 $\pm$ 33.6 mg L^{-1} 3-methyl butanol, 19.6 $\pm$ 0.2 to 44.6 $\pm$ 5.5 mg L^{-1} 2-methyl butanol, 2573.9 $\pm$ 402.8 to 3748.5 $\pm$ 462.0 μg L^{-1} hexanol and 12.1 $\pm$ 1.4 to 30.3 $\pm$ 3.7 mg L^{-1} 2-phenyl ethanol.

Acetic acid esters: Although commercial yeast strains and nutrient sources did not influence the formation of higher alcohols, all acetic acid ester productions were significantly different among the treatment combinations (**Table 4-10** and **Figure 4-19**). The acetic acid ethyl esters in this trial were identified by relative peak area as their concentrations were small and out of the limit of quantification (50 mg L^{-1}). Strain VIN7 was the highest acetic acid ethyl ester producer without response to the nutrient source (19.3 $\pm$ 2.5 and 22.3 $\pm$ 4.7 of relative peak area, respectively). The Sauvignon strain seemed to be the predominant producer of most acetic acid esters, excluding acetic acid hexyl ester, with less dependence on the nutrient source. These wines had the highest amounts of these esters; 444.3 $\pm$ 32.9 and 404.6 $\pm$ 15.7 μg L^{-1} acetic acid 3-methylbutyl ester, 36.4 $\pm$ 3.4 and 34.0 $\pm$ 1.1 μg L^{-1} acetic acid 2-methylbutyl ester and 80.4 $\pm$ 0.9 and 83.5 $\pm$ 7.7 μg L^{-1} acetic acid 2-phenylethyl ester. The concentration of acetic acid hexyl ester was most obvious in the VIN7 wines with both nutrient sources (144.7 $\pm$ 12.7 and 128.2 $\pm$ 9.6 μg L^{-1}, respectively), while acetic acid 2-phenylethyl ester and acetic acid 3- and 2-methylbutyl ester were highest in the Fermaid E sample (81.1 $\pm$ 9.6, 467.7 $\pm$ 100.9 and 29.7 $\pm$ 5.5 μg L^{-1}, respectively). Strain VIN13 also developed the highest amounts of acetic acid 2-methylbutyl ester in both nutrient sources (31.1 $\pm$ 3.7 and 31.6 $\pm$ 4.2 μg L^{-1}, respectively), acetic acid 3-methylbutyl ester in the Fermaid E treatment (341.3 $\pm$ 56.0 μg L^{-1}) and acetic acid 2-phenylethyl ester in the OptiWhite sample (68.2 $\pm$ 9.9 μg L^{-1}). Overall, most acetic acid esters tended to increase with the Fermaid E supplement, however it is strain-dependent.

Table 4-10 Concentration of higher alcohols and acetic acid ethyl ester found in finished Sauvignon blanc wines developed by nine yeast strains with two nutrient sources

Yeast	Nutrient	2-Methyl propanol (mg L^{-1})	3-Methyl butanol (mg L^{-1})	2-Methyl butanol (mg L^{-1})	Hexanol (μg L^{-1})	2-Phenyl ethanol (mg L^{-1})	Acetic acid ethyl ester (relative peak area)
EC1118	Fermaid E	29.0 ± 5.8 a	157.1 ± 16.1 a	28.0 ± 3.2 a	3411.3 ± 311.6 a	21.1 ± 0.2 a	12.4 ± 0.2 b
	OptiWhite	23.0 ± 9.8 a	111.6 ± 28.7 a	22.7 ± 7.7 a	2875.4 ± 478.2 a	21.8 ± 0.2 a	9.5 ± 2.1 bcde
Sauvignon	Fermaid E	28.0 ± 3.2 a	134.1 ± 11.3 a	33.6 ± 2.2 a	3209.5 ± 196.0 a	20.4 ± 2.3 a	7.9 ± 0.5 bcde
	OptiWhite	43.0 ± 6.0 a	170.6 ± 19.1 a	44.6 ± 5.5 a	3448.7 ± 245.2 a	30.2 ± 1.1 a	7.0 ± 1.0 cde
VL3	Fermaid E	29.5 ± 2.1 a	136.5 ± 7.8 a	30.4 ± 1.6 a	3054.4 ± 16.7 a	15.5 ± 0.6 a	11.3 ± 1.1 bcd
	OptiWhite	28.0 ± 2.5 a	114.1 ± 4.6 a	28.4 ± 1.7 a	2770.1 ± 101.7 a	16.8 ± 1.2 a	9.2 ± 0.9 bcde
X5	Fermaid E	24.5 ± 2.9 a	118.3 ± 13.1 a	27.7 ± 3.1 a	2580.1 ± 69.1 a	14.8 ± 0.9 a	9.2 ± 1.4 bcde
	OptiWhite	30.0 ± 1.8 a	122.7 ± 2.5 a	32.3 ± 0.1 a	2730.2 ± 16.3 a	17.1 ± 0.2 a	9.0 ± 0.6 bcde
VIN7	Fermaid E	21.5 ± 0.6 a	109.3 ± 2.4 a	19.6 ± 0.2 a	2926.2 ± 217.4 a	12.9 ± 0.5 a	19.3 ± 2.5 a
	OptiWhite	34.0 ± 8.3 a	103.8 ± 14.1 a	22.8 ± 3.9 a	3467.1 ± 271.5 a	12.1 ± 1.4 a	22.3 ± 4.7 a
VIN13	Fermaid E	22.5 ± 3.6 a	121.1 ± 22.1 a	28.7 ± 5.7 a	2625.8 ± 424.3 a	17.9 ± 3.5 a	7.2 ± 1.3 cde
	OptiWhite	23.0 ± 5.4 a	113.2 ± 23.8 a	29.8 ± 6.5 a	2573.9 ± 402.8 a	20.2 ± 3.1 a	6.7 ± 1.6 de
4F9	Fermaid E	17.0 ± 1.5 a	121.3 ± 0.5 a	21.4 ± 0.0 a	3012.2 ± 93.5 a	23.6 ± 2.8 a	5.4 ± 0.1 e
	OptiWhite	31.0 ± 5.9 a	159.3 ± 37.6 a	33.7 ± 3.3 a	3569.0 ± 692.2 a	30.3 ± 3.7 a	8.9 ± 3.7 bcde
LVCB	Fermaid E	21.5 ± 1.2 a	158.6 ± 10.8 a	26.3 ± 2.3 a	3349.6 ± 123.8 a	24.0 ± 0.1 a	8.0 ± 0.7 bcde
	OptiWhite	26.0 ± 4.3 a	176.3 ± 33.6 a	33.1 ± 6.6 a	3748.5 ± 462.0 a	30.0 ± 3.2 a	9.3 ± 1.8 bcde
AWRI R2	Fermaid E	31.5 ± 4.1 a	148.5 ± 24.5 a	35.7 ± 6.3 a	3497.7 ± 217.4 a	24.0 ± 0.5 a	7.7 ± 1.2 cde
	OptiWhite	47.5 ± 6.7 a	173.2 ± 33.6 a	44.3 ± 9.0 a	3675.3 ± 471.3 a	25.6 ± 5.8 a	11.4 ± 1.6 bc

Each value shows the mean ± standard deviation. Values displaying the same letters within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

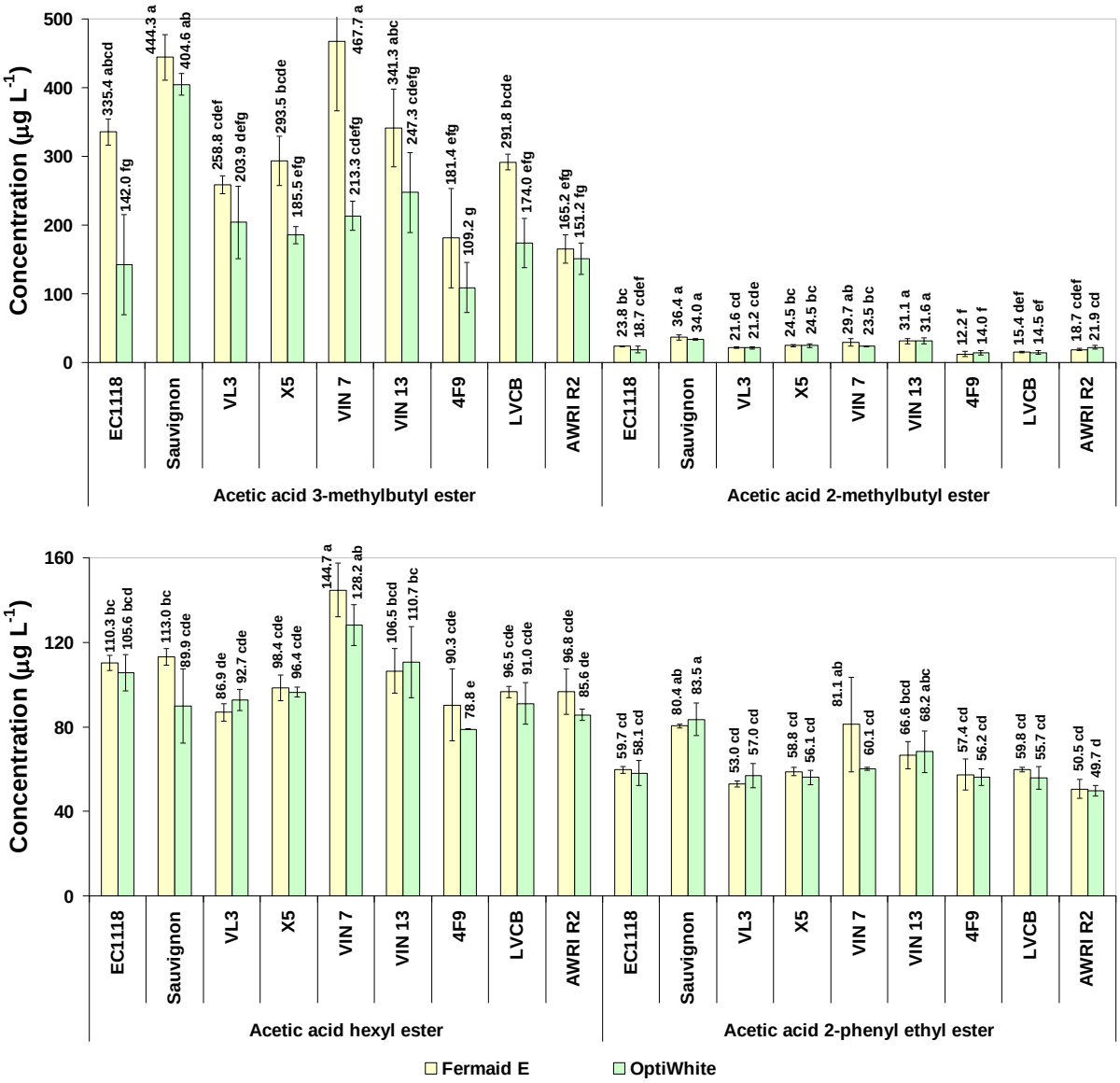


Figure 4-19 Concentration of acetic acid esters found in finished Sauvignon blanc wines developed by nine yeast strains with two nutrient sources

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

Ethyl and diethyl esters: **Table 4-11** shows that ethyl and diethyl ester productions differed according to treatment combinations of yeast strains and nutrient sources. The formation of LAEtE and BuEtE were similar among the wine treatments ranging from 19.9 ± 2.3 to 34.9 ± 5.0 mg L^{-1} and 116.0 ± 1.6 to 167.5 ± 2.5 μg L^{-1}, respectively. Nevertheless, strains VL3 and X5 tended to be the high producers of BuEtE, whereas strains EC1118, 4F9, LVCB and AWRI R2 tended to develop high amounts of LAEtE. The concentration of iBuEtE was most obvious in the AWRI R2 wines with either Fermaid E or OptiWhite addition (163.8 ± 6.1 and 174.4 ± 10.7 μg L^{-1}, respectively). Strain EC1118 was the highest producer of SAdiEtE in both nutrient sources (2268.5 ± 67.6 and 2321.6 ± 179.9 μg L^{-1}, respectively), whereas the highest amounts of PrEtE was present in the Fermaid E treatment (188.6 ± 21.0 μg L^{-1}). A high amount of SAdiEtE was produced by strains VL3 and VIN13 in both nutrient sources as well (2065.8 ± 195.5, 2351.1 ± 12.7 and 2044.0 ± 273.0, 2040.4 ± 191.9 μg L^{-1}, respectively). It is likely that most ethyl and diethyl esters were considerably more dependent on the yeast strain than on the nutrient source.

Monoterpenes: The result shows that the nine yeast strains had similar ability to release α-terpineol in any source of nutrient ranging from 12.5 ± 0.8 to 16.3 ± 0.1 μg L^{-1} (**Table 4-11**). While the other monoterpenes like *trans*-linalool oxide, *cis*-linalool oxide and linalool were detected only in trace quantities in all wine treatments (data not shown).

Medium-chain fatty acids and their ethyl esters: The results in **Table 4-12** and **Figure 4-20** demonstrate that the formation of OAEtE and DAEtE significantly differed among yeast strains and nutrient sources. The formation of MCFAs and HAEtE were similar among the wine treatments ranging from 5.5 ± 0.1 to 8.0 ± 0.1 mg L^{-1} hexanoic acid, 6.0 ± 0.1 to 9.2 ± 0.0 mg L^{-1} octanoic acid, 1.4 ± 0.3 to 3.0 ± 0.2 mg L^{-1} decanoic acid and 339.3 ± 29.6 to 551.3 ± 56.3 μg L^{-1} HAEtE. However, strain LVCB tended to be the high producer of hexanoic and octanoic acid. The addition of Fermaid E promoted the greatest production of OAEtE and DAEtE by the AWRI R2 strain (1067.7 ± 117.5 and 394.5 ± 61.8 μg L^{-1}, respectively). Strain VIN13 with the addition of Fermaid E also produced the highest amount of DAEtE (389.3 ± 53.7 μg L^{-1}).

Table 4-11 Concentration of ethyl, diethyl esters and α-terpineol found in finished Sauvignon blanc wines developed by nine yeast strains with two nutrient sources

Yeast	Nutrient	PrEtE (μg L^{-1})	BuEtE (μg L^{-1})	iBuEtE (μg L^{-1})	LAEtE (mg L^{-1})	SAdiEtE (μg L^{-1})	α-Terpineol (μg L^{-1})
EC1118	Fermaid E	188.6 ± 21.0 a	157.5 ± 14.8 a	75.6 ± 9.1 fgh	34.9 ± 5.0 a	2268.5 ± 67.6 ab	13.0 ± 0.1 a
	OptiWhite	133.3 ± 63.6 b	127.9 ± 15.2 a	61.5 ± 15.5 h	25.6 ± 7.0 a	2321.6 ± 179.9 a	13.6 ± 0.1 a
Sauvignon	Fermaid E	69.7 ± 3.7 f	156.1 ± 3.9 a	93.1 ± 8.3 efg	22.3 ± 1.6 a	1118.4 ± 122.4 f	15.7 ± 0.5 a
	OptiWhite	75.2 ± 12.5 ef	149.5 ± 14.7 a	107.5 ± 20.3 de	22.6 ± 1.7 a	1582.5± 20.4 de	14.4 ± 0.4 a
VL3	Fermaid E	94.6 ± 7.3 bcdef	167.5 ± 2.5 a	104.6 ± 5.1 e	24.7 ± 0.9 a	2065.8 ± 195.5 abc	14.1 ± 0.4 a
	OptiWhite	85.5 ± 15.7 cdef	158.1 ± 17.9 a	102.5 ± 16.8 ef	20.6 ± 0.2 a	2351.1 ± 12.7 a	14.0 ± 0.9 a
X5	Fermaid E	113.5 ± 5.0 bcdef	158.3 ± 3.4 a	131.6 ± 8.0 cd	27.7 ± 1.1 a	1828.6 ± 164.8 cde	14.2 ± 0.9 a
	OptiWhite	112.5 ± 8.2 bcdef	159.6 ± 8.7 a	139.9 ± 8.3 bc	25.2 ± 0.7 a	2009.8 ± 85.7 abc	14.8 ± 0.2 a
VIN7	Fermaid E	99.2 ± 6.5 bcdef	125.5 ± 14.2 a	74.6 ± 6.6 fgh	23.2 ± 1.4 a	1000.9 ± 157.1 f	16.3 ± 0.1 a
	OptiWhite	110.8 ± 12.6 bcdef	116.0 ± 1.6 a	76.1 ± 4.9 fgh	28.2 ± 3.6 a	1015.4 ± 63.4 f	15.1 ± 0.6 a
VIN13	Fermaid E	125.4 ± 11.1 bcd	146.7 ± 4.9 a	72.2 ± 1.5 gh	23.3 ± 2.3 a	2044.0 ± 273.0 abc	14.4 ± 0.2 a
	OptiWhite	116.0 ± 12.4 bcde	138.5 ± 4.5 a	70.4 ± 1.7 gh	19.9 ± 2.3 a	2040.4 ± 191.9 abc	14.6 ± 1.1 a
4F9	Fermaid E	113.1 ± 5.7 bcdef	131.4 ± 13.4 a	74.6 ± 11.3 fgh	27.2 ± 0.5 a	1822.2 ± 261.2 cde	15.1 ± 0.2 a
	OptiWhite	108.3 ± 12.7 bcdef	127.9 ± 9.3 a	90.8 ± 13.4 efg	28.0 ± 3.4 a	1884.7 ± 177.0 cd	14.1 ± 0.4 a
LVCB	Fermaid E	131.1 ± 14.6 bc	151.2 ± 8.7 a	72.0 ± 4.3 gh	30.3 ± 0.1 a	1785.4 ± 67.2 cde	15.1 ± 0.1 a
	OptiWhite	127.1 ± 17.5 bcd	149.0 ± 1.0 a	70.7 ± 1.2 gh	29.5 ± 3.7 a	1945.5 ± 32.4 bc	13.8 ± 0.6 a
AWRI R2	Fermaid E	82.8 ± 10.8 def	137.6 ± 0.5 a	163.8 ± 6.1 ab	30.3 ± 2.5 a	1744.9 ± 36.8 cde	15.1 ± 1.4 a
	OptiWhite	94.5 ± 7.1 bcdef	129.5 ± 0.3 a	174.4 ± 10.7 a	27.6 ± 2.6 a	1525.6 ± 126.9 e	12.5 ± 0.8 a

Each value shows the mean ± standard deviation. Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Table 4-12 Concentration of fatty acids found in finished Sauvignon blanc wines developed by nine yeast strains with two nutrient sources

Yeast	Nutrient	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})
EC1118	Fermaid E	6.2 ± 0.3 a	6.0 ± 1.0 a	1.7 ± 0.8 a
	OptiWhite	6.0 ± 0.0 a	6.8 ± 0.5 a	2.3 ± 0.3 a
Sauvignon	Fermaid E	6.6 ± 0.5 a	7.9 ± 0.3 a	2.9 ± 0.0 a
	OptiWhite	6.8 ± 0.0 a	7.0 ± 0.3 a	2.1 ± 0.6 a
VL3	Fermaid E	6.8 ± 0.4 a	7.2 ± 0.4 a	2.4 ± 0.2 a
	OptiWhite	6.3 ± 0.7 a	6.6 ± 0.5 a	1.9 ± 0.6 a
X5	Fermaid E	6.8 ± 0.1 a	7.5 ± 1.3 a	2.5 ± 0.7 a
	OptiWhite	6.7 ± 0.2 a	8.0 ± 0.3 a	3.0 ± 0.2 a
VIN7	Fermaid E	5.5 ± 0.1 a	6.0 ± 0.1 a	1.7 ± 0.6 a
	OptiWhite	5.9 ± 0.2 a	6.1 ± 0.0 a	2.0 ± 0.1 a
VIN13	Fermaid E	6.3 ± 0.3 a	7.6 ± 0.4 a	3.0 ± 0.5 a
	OptiWhite	6.2 ± 0.1 a	7.3 ± 0.4 a	2.7 ± 0.4 a
4F9	Fermaid E	7.1 ± 0.0 a	8.6 ± 0.5 a	2.8 ± 0.3 a
	OptiWhite	7.1 ± 0.4 a	7.8 ± 0.3 a	2.1 ± 0.6 a
LVCB	Fermaid E	8.0 ± 0.1 a	9.2 ± 0.0 a	2.9 ± 0.1 a
	OptiWhite	7.9 ± 0.0 a	8.7 ± 0.7 a	2.7 ± 0.3 a
AWRI R2	Fermaid E	7.7 ± 0.4 a	8.8 ± 0.9 a	3.0 ± 0.2 a
	OptiWhite	7.0 ± 0.3 a	7.0 ± 0.0 a	1.4 ± 0.3 a

Each value shows the mean ± standard deviation from two fermentation replicates. Values displaying the same letter (only ‘a’) within the same column and factor indicate no significant difference ($p>0.05$) among treatment combinations according to the DMRT test.

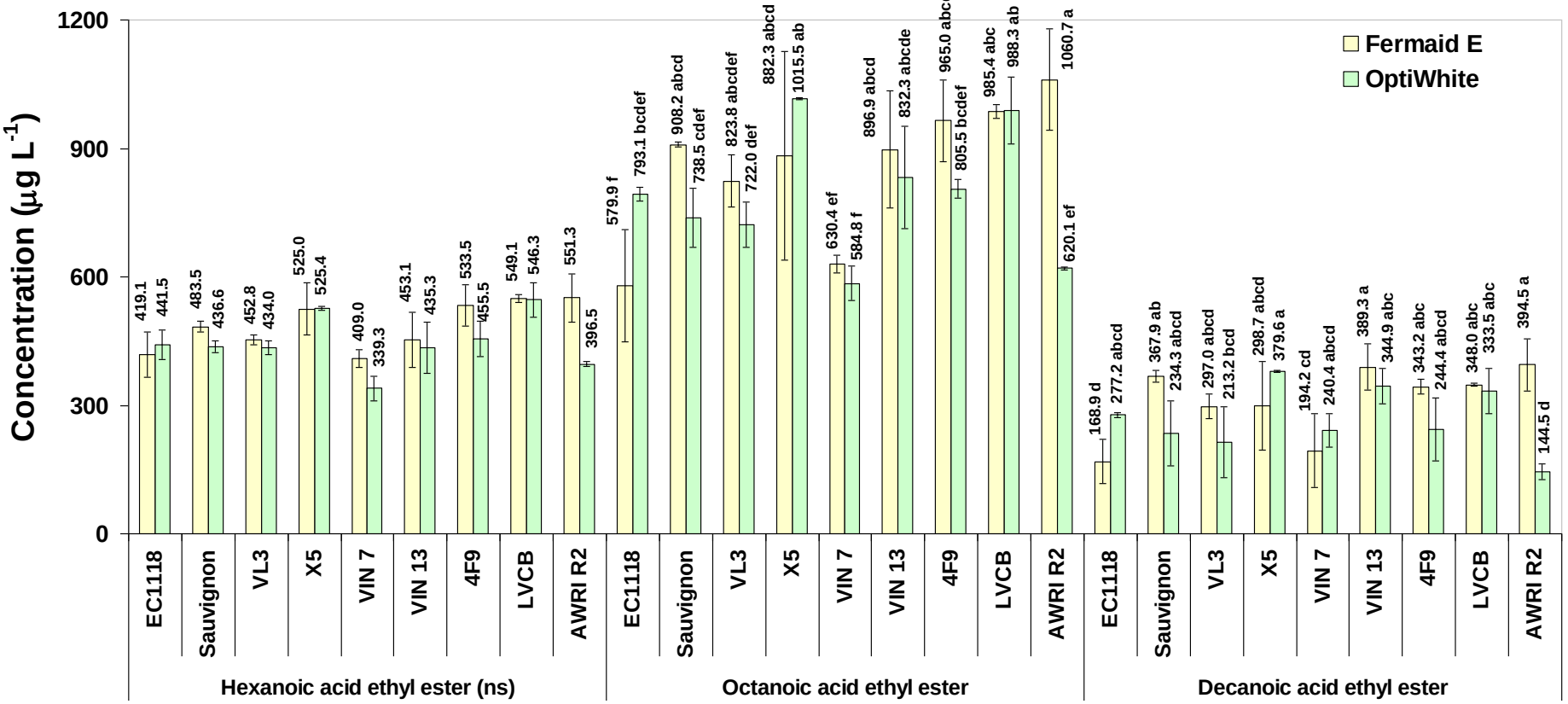


Figure 4-20 Concentration of fatty acid esters found in finished Sauvignon blanc wines developed by nine yeast strains with two nutrient sources

Vertical bars represent standard deviations from two fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test. Compounds displaying **"(ns)"** show no significant difference ($p>0.05$) among treatment combinations according to the DMRT test.

4.2.2 Impact of five commercial yeast strains and four nutrient sources on fermentative characteristics, metabolic compounds and wine aromas of Sauvignon blanc grape wines

In 2009, wine fermentations were conducted in fresh Sauvignon blanc juices. The fermentations were conducted using five commercial yeast strains of *Saccharomyces* (EC1118, X5, VIN7, Alchemy I and LittoLevure) and four commercial nutrient sources; 0.3 g L^{-1} Superstart, 0.4 g L^{-1} Fermaid O, 0.4 g L^{-1} Fermaid E blanc and 0.4 g L^{-1} Vitamon Ultra (VUltra). The yeast strains and nutrient sources were selected on the basis results, which were obtained from the previous trials of Scheurebe and Sauvignon blanc. The fermentation kinetics, some non-volatile and volatile compounds in final wines were investigated.

The growth kinetics: **Figure 4-21** shows that the EC1118 strain appeared to be the strongest strain followed by strain Alchemy I, while the LittoLevure seemed to be the weakest strain. The EC1118 strain had the fastest fermentation kinetic and duration in any kind of nutrient (15 days). Strain Alchemy I followed a similar pattern as strain EC1118, except in the presence of Fermaid O that caused slightly longer fermentation duration (16 days). On the other hand, strain LittoLevure had a longer lag phase and slow fermentation kinetics and times (26 days), particularly in either the Fermaid O or the Fermaid E treatment that seemed to result in a sluggish fermentation. However, the addition of Superstart and VUltra enhanced the growth kinetic of the latter strain and reduced the duration of fermentation to 21 days. Strain X5 tended to follow a similar pattern as strain LittoLevure, but had a slightly faster fermentation kinetic and time in respect to the Superstart and Fermaid E treatments (15 and 24 days, respectively). The VIN7 strain had the slow growth kinetic and long fermentation time in the Fermaid O fermentation (24 days), but addition of the other nutrient sources enhanced fermentation kinetics and time, particularly in the Superstart fermentation (17 days). In all cases, Supertart seemed to be the most effective nutrient source to accelerate fermentation kinetics, whereas Fermaid O tended to give the inverse response.

Chemical compositions: Commercial yeast strains and nutrient sources significantly affected the concentrations of residual sugar, but not glycerol (**Table 4-13**). The EC1118 and Alchemy I strains produced wines having low amounts of residual sugar and attained dryness ranging from 1.0 $\pm$ 0.2 to 2.4 $\pm$ 0.7 g L^{-1} and 0.6 $\pm$ 0.1 to 1.6 $\pm$ 0.3 g L^{-1}, respectively. On the contrary, strain LittoLevure produced wines having high amounts of residual sugar in the Fermaid O treatment (7.4 $\pm$ 0.1 g L^{-1}), followed by the VUltra treatment (4.5 $\pm$ 0.3 g L^{-1}).

The five yeast strains produced similar amounts of glycerol, which ranged from 2.0 ± 0.1 to 2.1 ± 0.0 g L^{-1} without any response to the nutrient source.

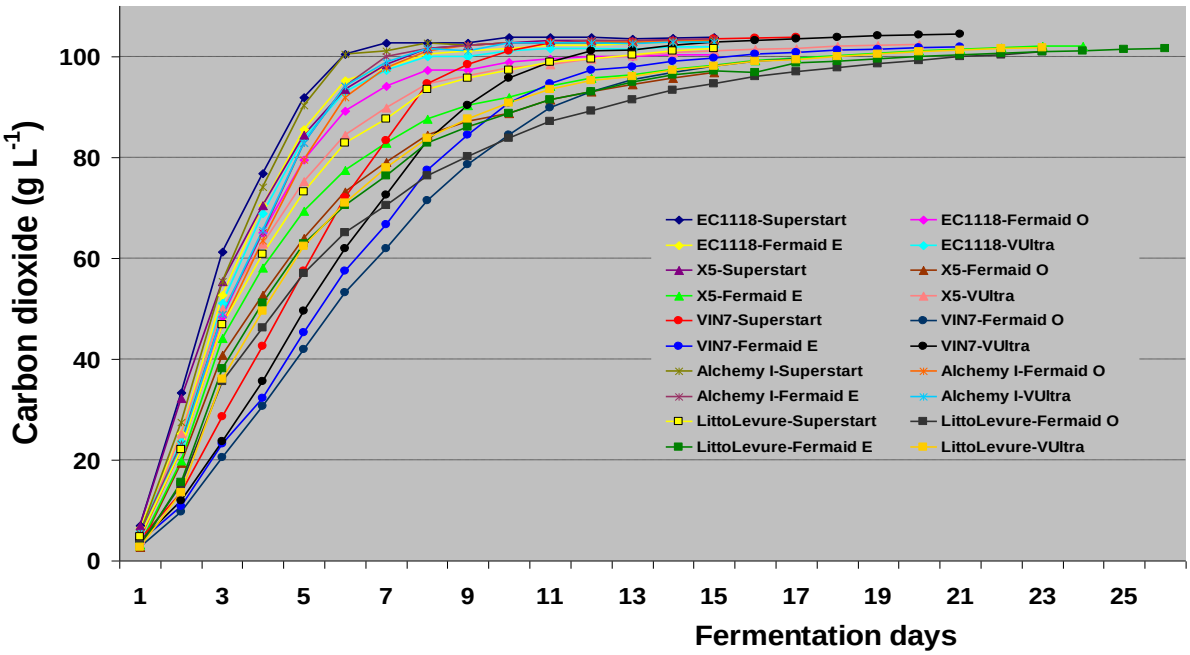


Figure 4-21 Fermentation kinetics of fresh Sauvignon blanc grape juice fermentations with five commercial yeast strains and four commercial nutrient sources

Organic acids: The results show that concentrations of citric acid were similar among the wine treatments ranging from 0.25 ± 0.00 to 0.27 ± 0.01 g L^{-1} (**Table 4-14**). When Superstart was added, strain EC1118 produced wine having the highest amount of lactic acid (0.31 ± 0.02 g L^{-1}) followed by strain Alchemy I (0.27 ± 0.02 g L^{-1}), while the VIN7 produced the greatest concentration of acetic acid (1.07 ± 0.03 g L^{-1}). Lactic acid was also most evident in the LittoLevure wine with the Fermaid O addition (0.30 ± 0.09 g L^{-1}). The LittoLevure strain produced wine with the highest amount of malic acid in the VUltra and Superstart treatments (3.23 ± 0.01 and 3.16 ± 0.04 g L^{-1}, respectively), shikimic acid in the Fermaid O and Fermaid E samples (22.26 ± 0.15 and 22.11 ± 0.27 mg L^{-1}, respectively) and tartaric acid in the VUltra treatment (3.38 ± 0.02 g L^{-1}). Strain X5 also produced wine having the greatest concentration of tartaric acid in the presence of Fermaid E (3.32 ± 0.01 g L^{-1}) and malic acid in the Fermaid O and VUltra samples (3.18 ± 0.04 and 3.16 ± 0.04 g L^{-1}). In addition, the VIN7 seemed to be the highest acetic acid producer, but the lowest producer of lactic acid.

Table 4-13 Chemical composition of finished wines obtained from fresh Sauvignon blanc grape juices fermented with five yeast strains and four nutrient sources

Yeast	Nutrient	Residual sugar (g L^{-1})	Glycerol (g L^{-1})
EC1118	Superstart	1.0 ± 0.2 fgh	2.0 ± 0.1 a
	Fermaid O	2.4 ± 0.7 d	2.0 ± 0.0 a
	Fermaid E	2.1 ± 0.8 de	2.0 ± 0.1 a
	VUltra	1.5 ± 0.1 def	2.0 ± 0.0 a
X5	Superstart	0.5 ± 0.2 h	2.0 ± 0.0 a
	Fermaid O	4.3 ± 0.6 bc	2.0 ± 0.0 a
	Fermaid E	4.9 ± 1.0 b	2.0 ± 0.1 a
	VUltra	0.7 ± 0.3 fgh	2.1 ± 0.0 a
VIN7	Superstart	1.2 ± 0.1 efgh	2.0 ± 0.1 a
	Fermaid O	3.9 ± 0.6 c	2.1 ± 0.1 a
	Fermaid E	3.8 ± 0.8 c	2.1 ± 0.1 a
	VUltra	2.1 ± 0.2 de	2.0 ± 0.0 a
Alchemy I	Superstart	0.8 ± 0.1 fgh	2.0 ± 0.1 a
	Fermaid O	1.6 ± 0.3 def	2.0 ± 0.0 a
	Fermaid E	0.7 ± 0.2 fgh	2.1 ± 0.0 a
	VUltra	0.6 ± 0.1 gh	2.0 ± 0.0 a
LittoLevure	Superstart	2.5 ± 0.4 d	2.1 ± 0.0 a
	Fermaid O	7.4 ± 0.1 a	2.1 ± 0.0 a
	Fermaid E	3.9 ± 0.3 c	2.1 ± 0.0 a
	VUltra	4.5 ± 0.3 bc	2.0 ± 0.0 a

Each value shows the mean ± standard deviation from three fermentation replicates .

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Table 4-14 Concentration of organic acids found in finished Sauvignon blanc wines produced by five yeast strains with four nutrient sources

Yeast	Nutrient	Tartaric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Lactic acid ($g\ L^{-1}$)	Shikimic acid ($mg\ L^{-1}$)	Acetic acid ($g\ L^{-1}$)	Citric acid ($g\ L^{-1}$)
EC1118	Superstart	3.31 ± 0.11 abc	3.00 ± 0.01 def	0.31 ± 0.02 a	20.65 ± 0.22 fgh	0.48 ± 0.01 ij	0.27 ± 0.01 a
	Fermaid O	3.20 ± 0.03 defg	3.07 ± 0.02 cd	0.23 ± 0.02 bcdef	20.64 ± 0.11 fgh	0.57 ± 0.01 g	0.26 ± 0.01 a
	Fermaid E	3.28 ± 0.07 bcde	3.05 ± 0.01 cd	0.25 ± 0.03 abcd	20.54 ± 0.09 ghi	0.54 ± 0.03 gh	0.26 ± 0.01 a
	VUltra	3.26 ± 0.03 bcde	3.04 ± 0.01 cde	0.22 ± 0.00 cdefg	20.37 ± 0.06 hij	0.53 ± 0.02 gh	0.25 ± 0.00 a
X5	Superstart	3.15 ± 0.06 fg	3.02 ± 0.01 de	0.23 ± 0.01 bcdef	20.70 ± 0.08 fgh	0.50 ± 0.01 hij	0.25 ± 0.01 a
	Fermaid O	3.05 ± 0.03 h	3.18 ± 0.04 ab	0.18 ± 0.03 efgh	21.27 ± 0.16 cd	0.40 ± 0.02 k	0.26 ± 0.01 a
	Fermaid E	3.32 ± 0.01 ab	3.11 ± 0.00 bc	0.21 ± 0.06 cdefgh	20.48 ± 0.15 hi	0.47 ± 0.03 j	0.26 ± 0.00 a
	VUltra	3.16 ± 0.04 fg	3.16 ± 0.04 ab	0.22 ± 0.02 cdefg	20.87 ± 0.37 efg	0.42 ± 0.03 k	0.27 ± 0.01 a
VIN7	Superstart	3.22 ± 0.01 cde	2.84 ± 0.01 g	0.16 ± 0.02 gh	19.60 ± 0.08 k	1.07 ± 0.03 a	0.27 ± 0.01 a
	Fermaid O	3.26 ± 0.01 bcde	2.95 ± 0.02 ef	0.16 ± 0.01 gh	20.27 ± 0.24 ij	1.03 ± 0.01 b	0.27 ± 0.01 a
	Fermaid E	3.22 ± 0.03 bcdef	2.96 ± 0.00 ef	0.16 ± 0.03 gh	20.11 ± 0.08 j	1.00 ± 0.02 bc	0.27 ± 0.01 a
	VUltra	3.18 ± 0.01 efg	2.93 ± 0.05 f	0.15 ± 0.05 h	20.05 ± 0.38 j	0.99 ± 0.03 c	0.27 ± 0.00 a
Alchemy I	Superstart	3.19 ± 0.02 efg	2.95 ± 0.03 ef	0.27 ± 0.02 abc	20.92 ± 0.23 def	0.52 ± 0.04 h	0.26 ± 0.00 a
	Fermaid O	3.21 ± 0.01 cdef	3.03 ± 0.02 cde	0.20 ± 0.01 defgh	21.17 ± 0.15 de	0.65 ± 0.02 ef	0.26 ± 0.00 a
	Fermaid E	3.31 ± 0.05 abc	3.02 ± 0.02 cde	0.22 ± 0.02 cdefg	20.98 ± 0.16 def	0.65 ± 0.01 ef	0.26 ± 0.00 a
	VUltra	3.30 ± 0.15 abcd	3.03 ± 0.01 cde	0.24 ± 0.03 bcde	20.98 ± 0.12 def	0.62 ± 0.03 f	0.25 ± 0.00 a
LittoLevure	Superstart	3.23 ± 0.04 bcde	3.16 ± 0.04 ab	0.22 ± 0.04 cdefg	21.54 ± 0.14 bc	0.66 ± 0.01 e	0.26 ± 0.01 a
	Fermaid O	3.10 ± 0.02 gh	2.98 ± 0.17 def	0.30 ± 0.09 ab	22.26 ± 0.15 a	0.51 ± 0.01 hi	0.26 ± 0.01 a
	Fermaid E	3.18 ± 0.01 efg	3.07 ± 0.06 cd	0.22 ± 0.03 cdefg	22.11 ± 0.27 a	0.48 ± 0.01 ij	0.26 ± 0.00 a
	VUltra	3.38 ± 0.02 a	3.23 ± 0.01 a	0.17 ± 0.00 fgh	21.79 ± 0.11 b	0.72 ± 0.01 d	0.26 ± 0.01 a

Each value shows the mean ± standard deviation. Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

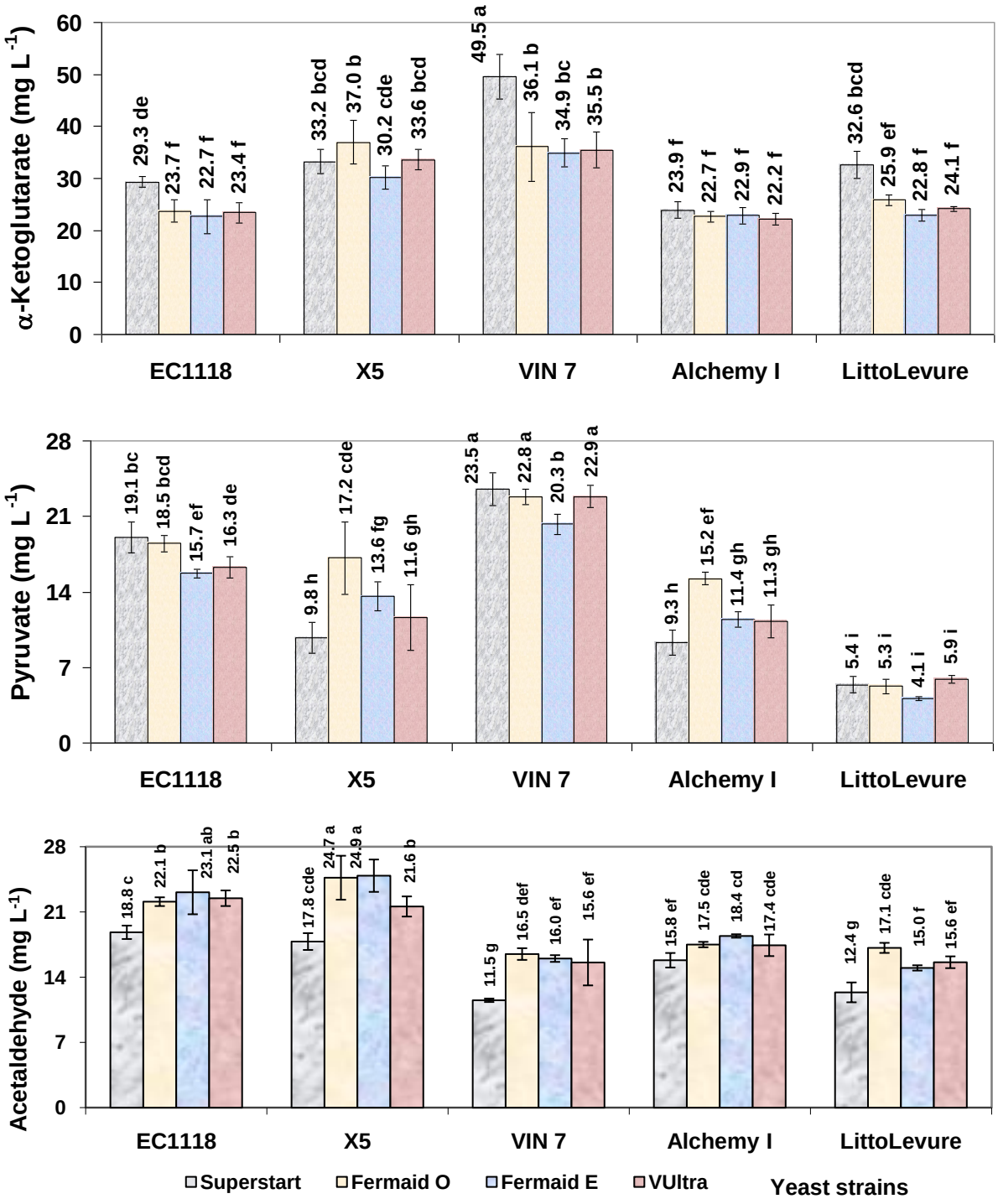


Figure 4-22 Concentration of SO_2-binding compounds found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

SO_2-binding compounds: As shown in **Figure 4-22**, the productions of these compounds by the five yeast strains were significantly different depending on the nutrient source. Strain VIN7 produced the greatest amount of pyruvate in most nutrient sources (22.8 ± 0.7 to 23.5 ± 1.5 mg L^{-1}), except in the Fermaid E sample (20.3 ± 0.7 mg L^{-1}), and α-ketoglutarate in the Superstart sample (49.5 ± 4.2 mg L^{-1}). The greatest concentration of acetaldehyde was produced by strain X5 in the Fermaid O and Fermaid E treatments (24.7 ± 2.4 and 24.9 ± 1.7 mg L^{-1}) and by strain EC1118 in the Fermaid E sample (23.1 ± 2.4 mg L^{-1}). Moreover, the addition of Superstart significantly decreased acetaldehyde formation.

Sulphur containing compounds: **Figure 4-23** and **4-24** show that the interaction of five commercial yeast strains and nutrient sources significantly only affected the concentration of SO_2 and CS_2. The concentrations of H_2S and DMS were not statistically different among all treatment combinations that ranging from 3.6 ± 1.0 to 5.7 ± 1.2 μg L^{-1} and 2.4 ± 0.1 to 3.0 ± 0.2 μg L^{-1}, respectively. The X5 strain was the dominant SO_2 forming producer with less response to the nutrient source (26.2 ± 0.2 to 28.9 ± 4.3 μg L^{-1}), while strain EC1118 produced the greatest amount of this compound in the Fermaid E and VUltra treatments (28.6 ± 1.9 and 30.7 ± 3.8 μg L^{-1}, respectively). The addition of Fermaid E promoted the greatest production of CS_2 by strains LittoLevure and X5 (11.1 ± 2.1 and 10.5 ± 2.2 μg L^{-1}, respectively).

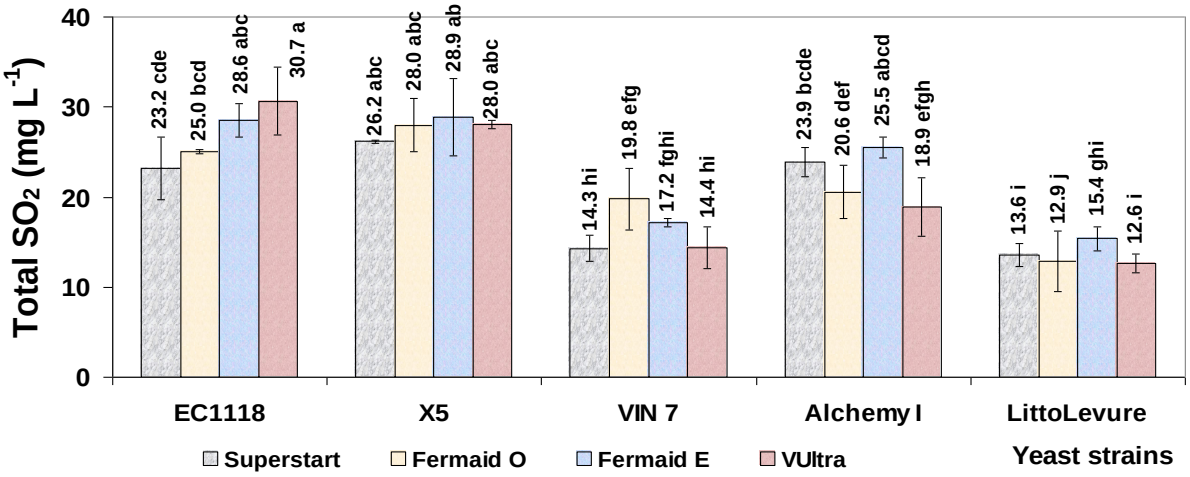


Figure 4-23 Concentration of total sulphur dioxide found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

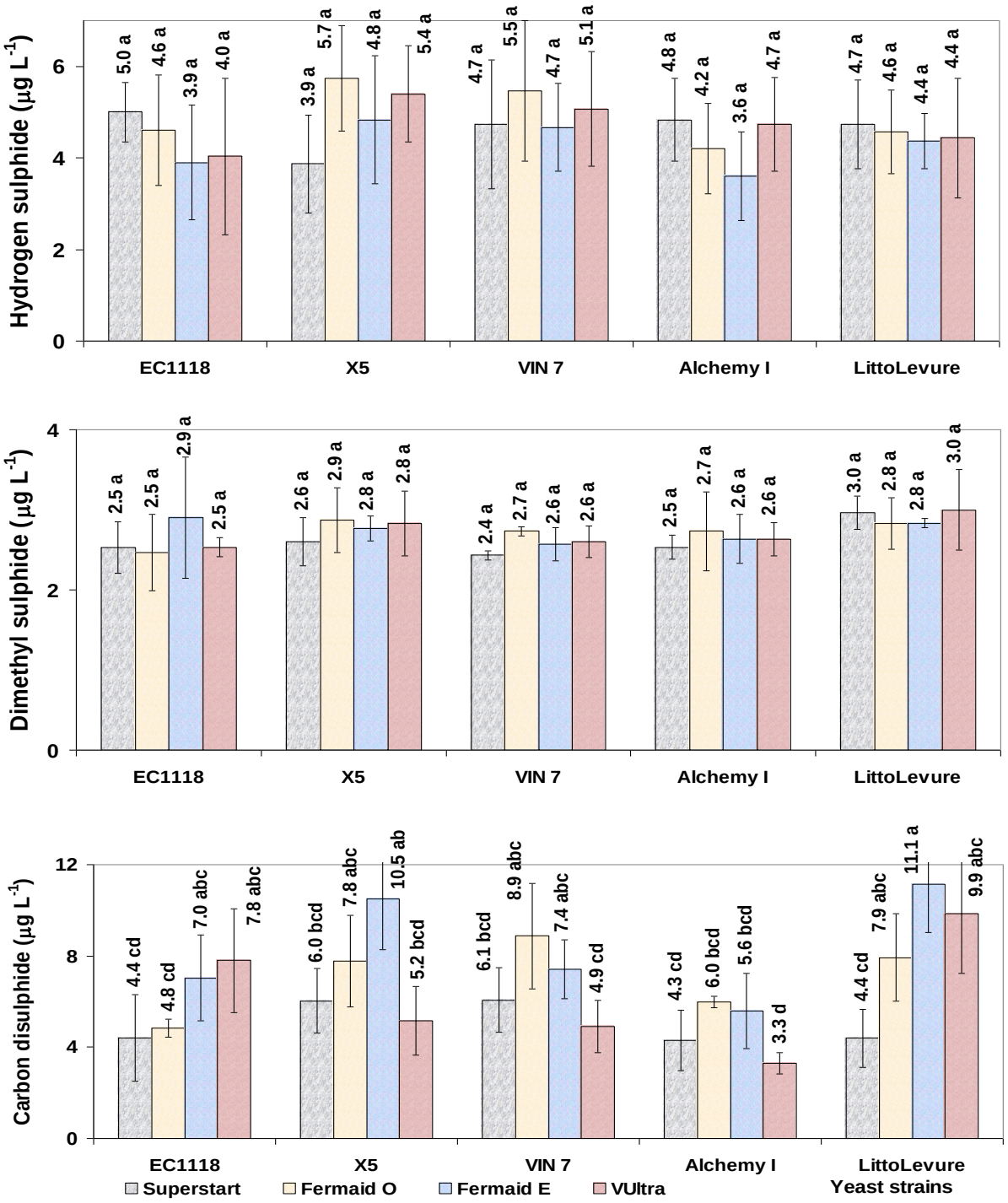


Figure 4-24 Concentration of sulphur containing compounds found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$), whereas those displaying the same letters are not significantly different ($p>0.05$) according to the DMRT test.

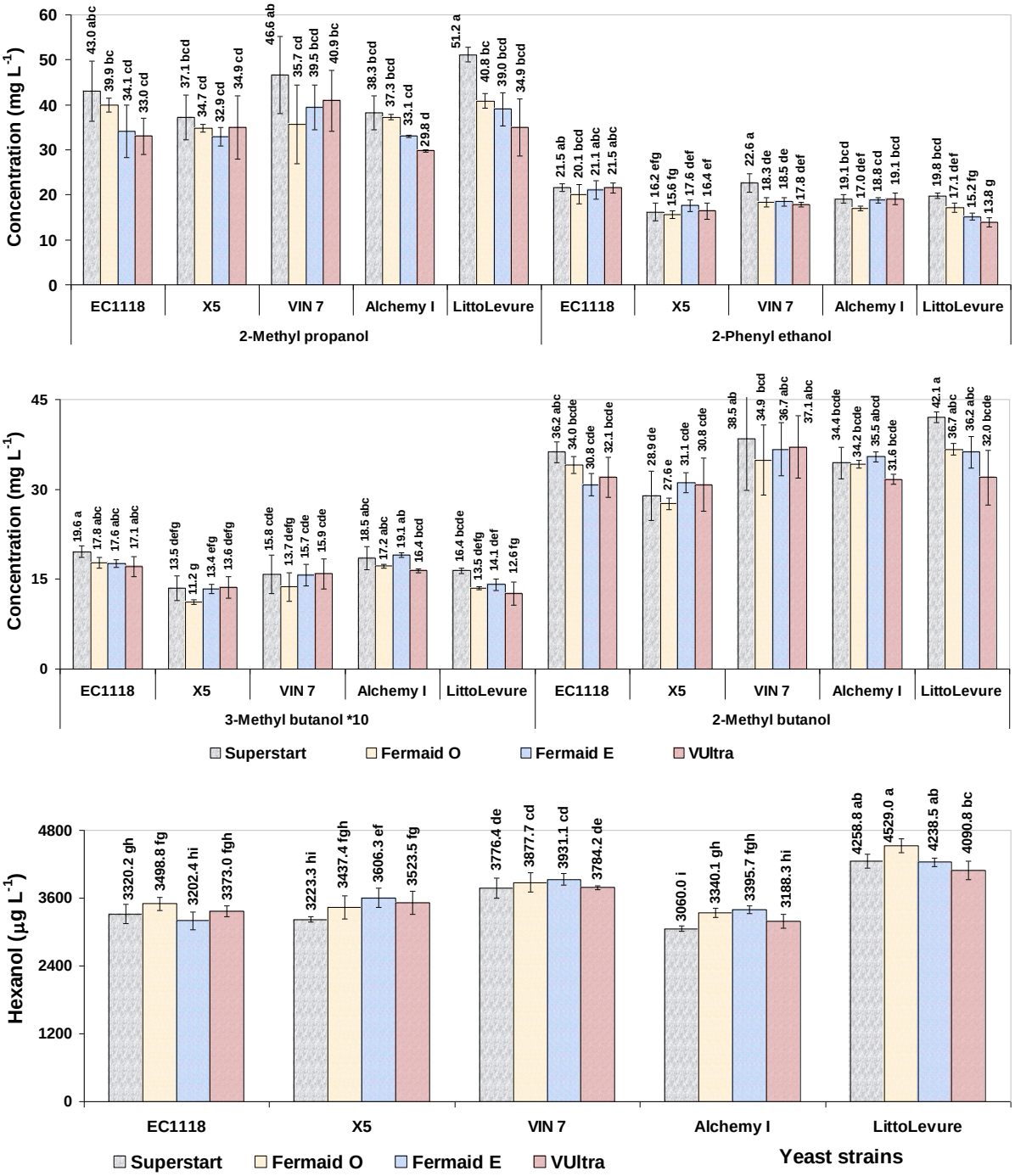


Figure 4-25 Concentration of higher alcohols found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Higher alcohols: The influence of five commercial yeast strains on higher alcohol production was dependent upon the nitrogen source supplemented (**Figure 4-25**). Strain EC1118 was the high producer of 3-methyl butanol and 2-phenyl ethanol with less response to the nutrient source (171.4 ± 16.7 to 196.0 ± 10.2 mg L^{-1} and 20.1 ± 2.0 to 21.5 ± 0.8 mg L^{-1}). Strain Alchemy I followed a similar pattern of 3-methyl butanol production (164.4 ± 3.2 to 190.6 ± 4.2 mg L^{-1}). Strain LittoLevure was the most hexanol producer in any kind of nutrient (4090.8 ± 164.3 to 4529.0 ± 121.3 μg L^{-1}), while the greatest amounts of 2-methyl propanol and 2-methyl butanol were produced in the Superstart variant (51.2 ± 1.6 and 42.1 ± 0.9 mg L^{-1}, respectively). The Superstart treatment also promoted the highest formation of 2-methyl propanol and 2-methyl butanol by strain VIN7 (46.6 ± 8.6 and 38.5 ± 8.6 mg L^{-1}, respectively).

Acetic acid esters: **Figure 4-26** shows that strain Alchemy I was the most predominant producer of acetic acid hexyl ester and acetic acid ethyl ester with less response to the nutrient source (536.8 ± 49.2 to 576.1 ± 34.4 μg L^{-1} and 212.8 ± 21.1 to 289.4 ± 10.5 mg L^{-1}, respectively). Strain VIN7 was also the greatest producer of acetic acid ethyl ester in most nutrient sources (213.7 ± 27.5 to 273.5 ± 42.9 mg L^{-1}, respectively) although its concentration was slightly lower in the Fermaid O and Fermaid E samples. Acetic acid 3- and 2-methylbutyl ester and acetic acid 2-phenyl ethyl ester were also most evident in the Alchemy I wine with the Superstart supplementation (2960.8 ± 239.2, 115.3 ± 6.3 and 410.6 ± 4.3 μg L^{-1}, respectively). The presence of Superstart also stimulated strain EC1118 to produce the highest amount of acetic acid 2-methylbutyl ester (103.0 ± 16.0 μg L^{-1}).

Ethyl and diethyl esters: As shown in **Table 4-15**, the production of ethyl esters significantly differed depending on the yeast strain and the nitrogen source, while succinic acid diethyl esters were detected in all wine treatments only in trace quantities (data not shown). Strain EC1118 developed the greatest amount of PrEtE in the presence of Superstart (98.8 ± 11.5 μg L^{-1}), whereas BuEtE was most evident in the Superstart and Fermaid O samples (366.4 ± 19.4 and 380.1 ± 12.7 μg L^{-1}, respectively) and also in the X5 wine with Superstart addition (361.1 ± 39.5 μg L^{-1}). Strain Alchemy I produced the greatest amount of iBuEtE in the Fermaid O and Fermaid E samples (13.5 ± 0.5 and 13.3 ± 0.8 μg L^{-1}, respectively) and PrEtE in the presence of Fermaid E (92.5 ± 5.2 μg L^{-1}). The greatest amount of iBuEtE was also produced by strain LittoLevure in the Superstart treatment (12.2 ± 0.3 μg L^{-1}), while LAEtE was most evident in the Fermaid O sample (14.4 ± 2.0 mg L^{-1}). Strain VIN7 seemed to be the low producer of BuEtE and LAEtE, while the LittoLevure was the low PrEtE producer with less response to nutrient source.

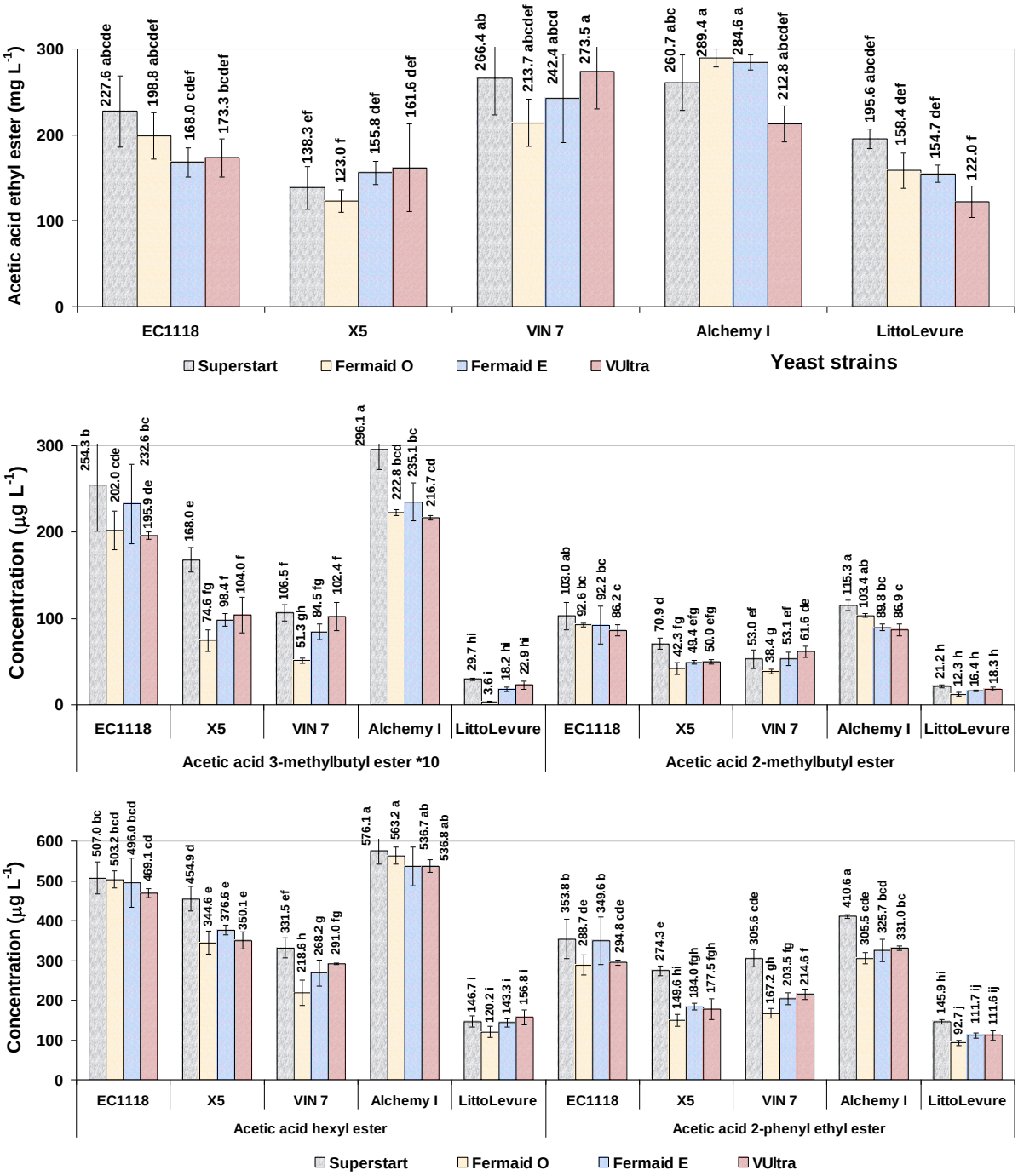


Figure 4-26 Concentration of acetic acid esters found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Table 4-15 Concentration of ethyl esters found in finished Sauvignon blanc grape wines developed by five yeast strains with four nutrient sources

Yeast	Nutrient	PrEtE ($\mu g\ L^{-1}$)	BuEtE ($\mu g\ L^{-1}$)	iBuEtE ($\mu g\ L^{-1}$)	LAEtE ($mg\ L^{-1}$)
EC1118	Superstart	98.8 ± 11.5 a	366.4 ± 19.4 ab	9.6 ± 1.3 c	11.0 ± 0.8 bcd
	Fermaid O	80.5 ± 5.0 bc	380.1 ± 12.7 a	8.8 ± 0.5 cd	9.9 ± 0.4 bcde
	Fermaid E	83.6 ± 15.7 abc	351.3 ± 18.1 abc	9.0 ± 2.1 cd	10.4 ± 0.5 bcde
	VUltra	79.5 ± 11.1 bc	327.7 ± 24.4 c	7.2 ± 0.7 de	10.2 ± 0.2 bcde
X5	Superstart	58.2 ± 12.5 de	361.1 ± 39.5 ab	10.0 ± 1.3 bc	10.1 ± 0.3 bcde
	Fermaid O	52.6 ± 4.6 ef	326.2 ± 19.5 c	10.2 ± 0.6 bc	9.3 ± 0.1 cde
	Fermaid E	62.0 ± 6.8 de	338.5 ± 8.7 bc	9.2 ± 0.6 cd	10.0 ± 0.4 bcde
	VUltra	60.4 ± 8.6 de	338.4 ± 9.3 bc	8.8 ± 1.1 cd	10.1 ± 0.6 bcde
VIN7	Superstart	48.4 ± 14.5 def	176.9 ± 29.1 f	9.3 ± 2.2 c	8.5 ± 0.4 e
	Fermaid O	39.3 ± 6.9 fg	170.6 ± 13.4 f	8.5 ± 2.0 cde	8.6 ± 0.4 de
	Fermaid E	47.1 ± 6.3 ef	177.6 ± 8.4 f	8.9 ± 1.4 cd	8.7 ± 0.3 de
	VUltra	50.8 ± 9.7 ef	180.8 ± 17.7 f	9.0 ± 2.0 cd	9.0 ± 0.5 de
Alchemy I	Superstart	86.1 ± 8.2 abc	325.7 ± 17.1 c	9.0 ± 0.4 cd	11.0 ± 0.3 bcd
	Fermaid O	84.3 ± 1.4 abc	324.7 ± 5.0 c	13.5 ± 0.5 a	11.3 ± 0.0 bc
	Fermaid E	92.5 ± 5.2 ab	325.4 ± 10.3 c	13.3 ± 0.8 a	12.1 ± 0.2 b
	VUltra	71.1 ± 5.2 cd	289.9 ± 4.6 c	6.0 ± 0.7 e	9.8 ± 0.3 bcde
LittoLevure	Superstart	37.6 ± 2.6 fgh	336.1 ± 11.2 bc	12.2 ± 0.3 ab	10.4 ± 0.4 bcde
	Fermaid O	22.9 ± 1.7 h	248.1 ± 7.0 e	10.0 ± 0.3 bc	14.4 ± 2.0 a
	Fermaid E	29.3 ± 2.9 gh	264.2 ± 5.1 de	10.2 ± 0.4 bc	11.7 ± 1.9 b
	VUltra	25.1 ± 7.0 gh	259.5 ± 11.3 e	8.2 ± 1.3 cde	10.1 ± 0.3 bcde

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Medium-chain fatty acids and their ethyl esters: **Table 4-16** and **Figure 4-27** show that the EC1118 strain was the greatest producer of MCFAs, hexanoic acid (7.4 ± 0.3 to 7.7 ± 0.5 mg L^{-1}), octanoic acid (6.9 ± 0.4 to 7.2 ± 0.2 mg L^{-1}) and decanoic acid (1.5 ± 0.0 to 1.9 ± 0.1 mg L^{-1}) with less response to the nutrient source. It also developed the highest concentrations of HAEtE (876.3 ± 14.3 to 959.0 ± 39.4 $\mu g\ L^{-1}$), OAEtE (999.8 ± 101.6 to 1114.1 ± 12.4 $\mu g\ L^{-1}$) and DAEtE (485.7 ± 45.2 to 536.2 ± 64.8 $\mu g\ L^{-1}$). The X5 strain was also the most forming strain of MCFAs, 7.6 ± 0.1 to 8.1 ± 0.6 mg L^{-1} hexanoic acid, 6.4 ± 0.1 to 7.1 ± 0.3 mg L^{-1} octanoic acid and 1.6 ± 0.1 to 1.8 ± 0.2 mg L^{-1} hexanoic acid. The highest formation of OAEtE and DAEtE was also most evident in the X5 wines with any source of

nutrient (952.1 ± 97.4 to 1080.1 ± 36.1 μg L^{-1} and 487.9 ± 17.4 to 501.2 ± 49.4 μg L^{-1}, respectively). On the other hand, strain VIN7 was the lowest producer of MCFAs and their related ethyl esters.

Monoterpenes: Surprisingly, under the condition in this trial, all monoterpenes, α-terpineol, *trans*-linalool oxide, *cis*-linalool oxide and linalool were not detected in all wine treatments (data not shown).

Table 4-16 Concentration of medium-chain fatty acids found in finished Sauvignon blanc wines developed by five yeast strains with four nutrient sources

Yeast	Nutrient	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})
EC1118	Superstart	7.6 ± 0.2 ab	6.9 ± 0.4 a	1.5 ± 0.0 bc
	Fermaid O	7.7 ± 0.5 ab	7.0 ± 0.4 a	1.7 ± 0.2 ab
	Fermaid E	7.4 ± 0.3 ab	7.2 ± 0.2 a	1.9 ± 0.0 a
	VUltra	7.6 ± 0.2 ab	7.0 ± 0.2 a	1.9 ± 0.1 a
X5	Superstart	8.1 ± 0.6 a	7.1 ± 0.3 a	1.8 ± 0.2 ab
	Fermaid O	7.6 ± 0.1 ab	6.4 ± 0.1 abc	1.7 ± 0.0 ab
	Fermaid E	7.9 ± 0.4 a	6.5 ± 0.3 ab	1.6 ± 0.1 ab
	VUltra	7.9 ± 0.2 a	6.7 ± 0.3 ab	1.7 ± 0.1 ab
VIN7	Superstart	5.2 ± 0.4 e	3.8 ± 0.4 de	0.8 ± 0.2 d
	Fermaid O	5.3 ± 0.3 e	3.9 ± 0.3 de	0.9 ± 0.3 d
	Fermaid E	5.4 ± 0.1 e	3.9 ± 0.3 de	0.8 ± 0.2 d
	VUltra	5.2 ± 0.4 e	3.7 ± 0.3 de	0.8 ± 0.2 d
Alchemy I	Superstart	6.5 ± 0.2 cd	5.6 ± 0.1 c	1.3 ± 0.0 c
	Fermaid O	5.8 ± 0.2 de	4.3 ± 0.2 de	0.6 ± 0.1 d
	Fermaid E	5.8 ± 0.1 de	4.4 ± 0.2 d	0.6 ± 0.0 d
	VUltra	6.6 ± 0.2 cd	5.9 ± 0.3 bc	1.5 ± 0.2 bc
LittoLevure	Superstart	6.7 ± 0.2 cd	5.7 ± 0.2 c	1.2 ± 0.1 c
	Fermaid O	7.0 ± 0.2 bc	5.9 ± 0.3 bc	1.3 ± 0.1 c
	Fermaid E	7.0 ± 0.3 bc	5.8 ± 0.5 bc	1.3 ± 0.3 c
	VUltra	6.9 ± 0.3 bc	5.7 ± 0.4 c	1.3 ± 0.3 c

Each value shows the mean ± standard deviation from three fermentation replicates. Values displaying different letters within the same column are significantly different ($p<0.05$) among treatments according to the DMRT test.

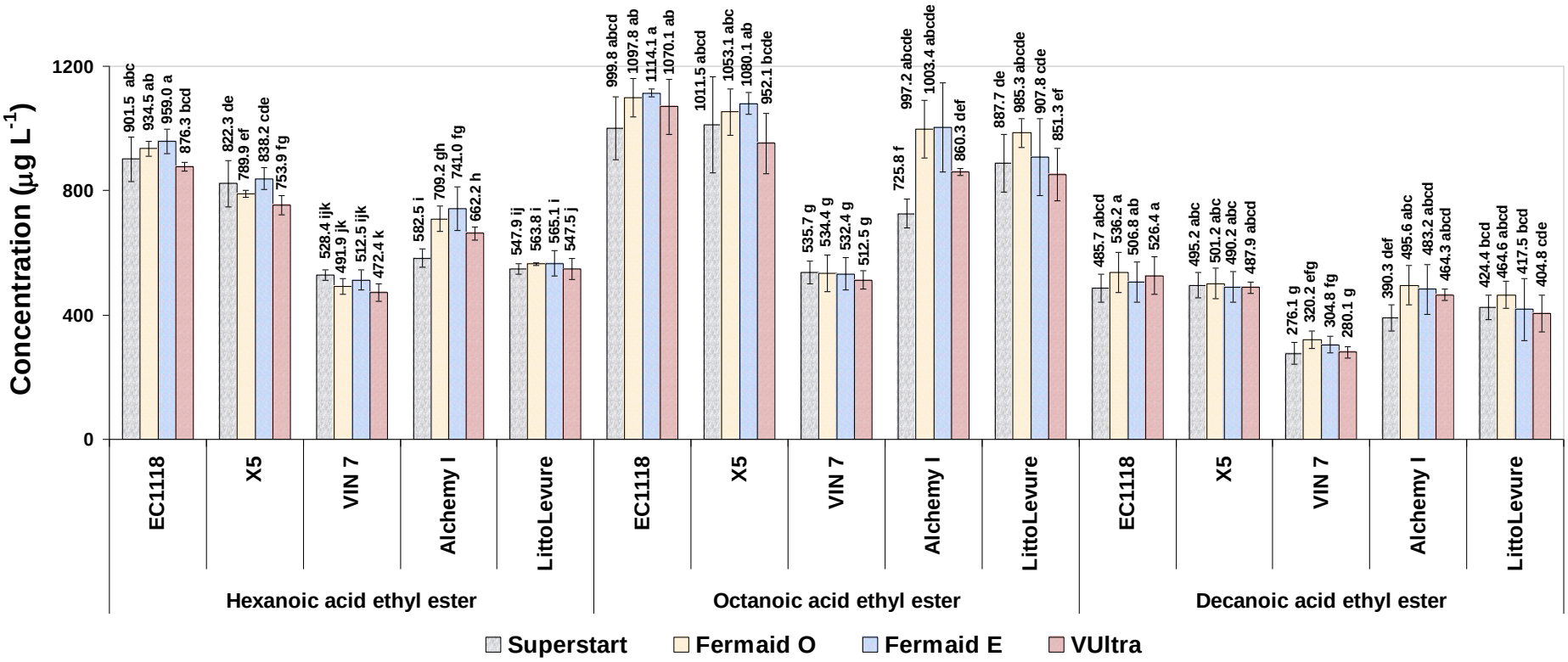


Figure 4-27 Concentration of fatty acid ethyl esters found in finished Sauvignon blanc grape wines produced by five yeast strains with four nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

4.3 Effects of yeast strains and nutrient supplements on wine fermentation and quality of yellow passion fruit wines

The experimental results which are described in the following chapters were obtained from four trials of yellow passion fruit (YPF) juice fermented with different commercial *Saccharomyces* yeast strains and/or nutrient sources and concentrations.

4.3.1 Effect of fourteen commercial *Saccharomyces* yeast strains on fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

In 2009, the fermentation trial was performed in yellow passion fruit (YPF) juice. Fourteen commercial yeast strains; EC1118, Sauvignon, VL3, X5, X16, VIN13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2, QA23 and Freddo were used in fermentation of this YPF trial. These commercial yeast strains were obtained from the basis results of the Sauvignon blanc and Scheurebe grape wines. The fermentation kinetics, some metabolic and aromatic compounds in obtained YPF wines were investigated.

Fermentation kinetics: The results show that the fourteen commercial yeast strains had significantly different fermentation kinetics (**Figure 4-28**). The VIN13, LVCB and QA23 had the fastest growth kinetics and the shortest fermentation times for 20 days, followed by strains EC1118, Alchemy I and II, X5, 4F9 and Freddo (21 days). While, strain Sauvignon had a long lag phase (5 days) and resulted in a sluggish fermentation kinetic and duration (28 days), strain AWRI R2 followed similar pattern, however had shorter lag phase (4 days). Nevertheless, the fourteen yeast strains completed fermentations and reached dryness with low residual sugar below 4 g L^{-1} (**Figure 4-29**).

Chemical composition: As shown in **Figure 4-29**, strain X16 produced the highest amount of glycerol in YPF wine (6.0 $\pm$ 0.0 g L^{-1}), followed by the LittoLevure and AWRI R2 (5.9 $\pm$ 0.2 and 5.7 $\pm$ 0.1 g L^{-1}, respectively). On the other hand, the EC1118 strain was the lowest glycerol producer as well as strain X5 (4.9 $\pm$ 0.2 and 5.0 $\pm$ 0.1 g L^{-1}, respectively). The YPF fermentations with either Alchemy I or X16 strain had the highest amounts of residual sugar (1.5 $\pm$ 0.2 and 1.3 $\pm$ 0.0 g L^{-1}, respectively). However, their concentrations resulted in dryness. While, the QA23, LVCB, 4F9 and AWRI R2 strains produced YPF wines having low amounts of residual sugar (0.3 $\pm$ 0.1, 0.4 $\pm$ 0.1, 0.5 $\pm$ 0.1 and 0.4 $\pm$ 0.1 g L^{-1}, respectively).

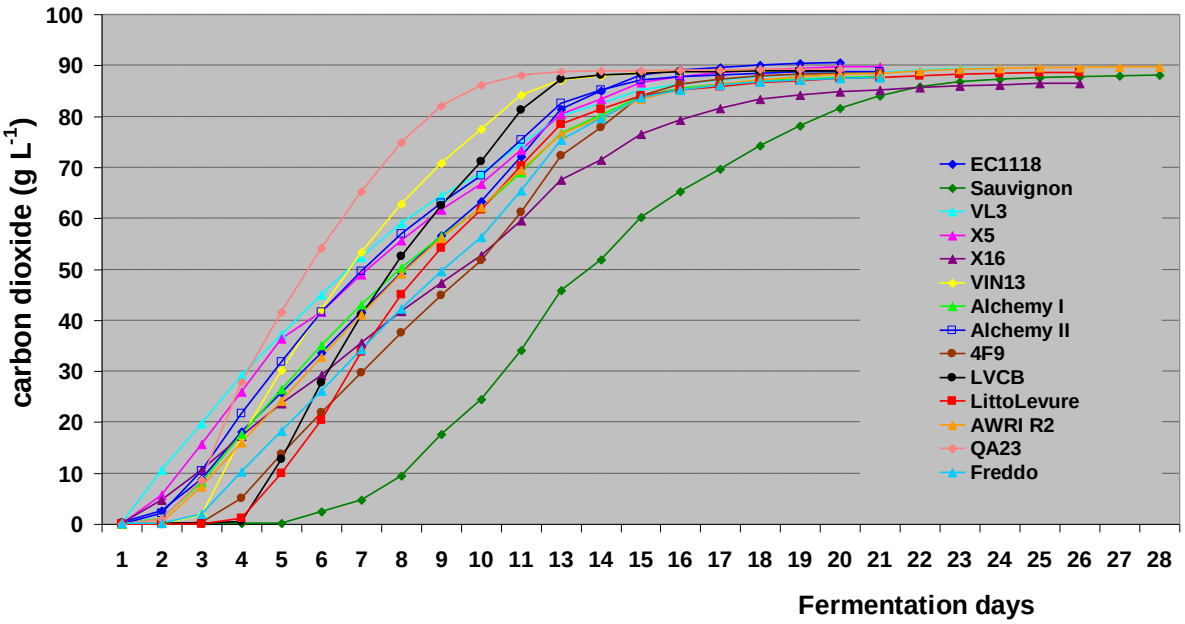


Figure 4-28 Growth kinetics of YPF juice fermentations with 14 different commercial yeast strains

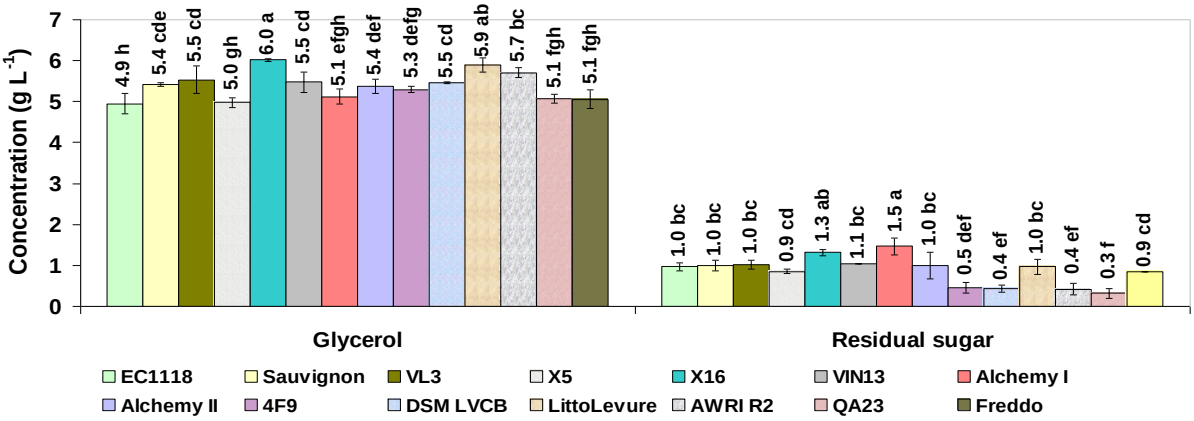


Figure 4-29 Concentration of residual sugar and glycerol in finished YPF wines produced by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each compound according to the DMRT test.

Table 4-17 Concentration of organic acids found in finished YPF wines produced by 14 yeast strains

Yeast	Citric acid (g L^{-1})	Malic acid (g L^{-1})	Acetic acid (g L^{-1})
EC1118	3.63 ± 0.04 a	0.68 ± 0.01 de	0.29 ± 0.02 cd
Sauvignon	3.67 ± 0.02 a	0.91 ± 0.11 b	0.22 ± 0.01 fg
VL3	3.67 ± 0.02 a	0.74 ± 0.03 de	0.26 ± 0.01 de
X5	3.62 ± 0.02 a	0.66 ± 0.01 ef	0.29 ± 0.01 cd
X16	3.65 ± 0.03 a	1.15 ± 0.03 a	0.26 ± 0.01 de
VIN13	3.61 ± 0.07 a	0.75 ± 0.02 d	0.31 ± 0.01 c
Alchemy I	3.62 ± 0.03 a	0.55 ± 0.01 g	0.39 ± 0.01 a
Alchemy II	3.61 ± 0.01 a	0.56 ± 0.01 g	0.39 ± 0.01 a
4F9	3.54 ± 0.01 a	0.91 ± 0.01 b	0.23 ± 0.01 fg
LVCB	3.59 ± 0.03 a	0.59 ± 0.03 fg	0.35 ± 0.01 b
LittoLevure	3.60 ± 0.02 a	0.83 ± 0.11 c	0.24 ± 0.03 ef
AWRI R2	3.65 ± 0.01 a	0.67 ± 0.03 def	0.38 ± 0.03 a
QA23	3.64 ± 0.08 a	0.52 ± 0.01 g	0.27 ± 0.01 de
Freddo	3.59 ± 0.02 a	0.70 ± 0.03 de	0.21 ± 0.02 g

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$), whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Organic acids: The data in **Table 4-17** show that concentrations of citric acid were similar among the YPF wines ranging from 3.54 ± 0.01 to 3.67 ± 0.02 g L^{-1}. Whereas, the other organic acids like lactic, tartaric and shkimic acid were not detected in all YPF wines. Concentrations of malic acid were most evident in the YPF fermentation with X16 (1.15 ± 0.03 g L^{-1}), followed by strains Sauvignon and 4F9 (0.91 ± 0.11 to 0.91 ± 0.01 g L^{-1}, respectively), but less present in YPF wines of Alchemy I, II and QA23. In addition, the X16 strain produced about two-fold of initial malic acid amounts in the YPF juice, whereas strains 4F9 and Sauvignon catalyzed approximately 80 %. Strains Alchemy I and II and AWRI R2 were the highest acetic acid producers (0.39 ± 0.01, 0.39 ± 0.01 and 0.38 ± 0.03 g L^{-1}, respectively), while strains Sauvignon, 4F9 and Freddo were the lowest producers (0.22 ± 0.01, 0.23 ± 0.01 and 0.21 ± 0.02 g L^{-1}, respectively).

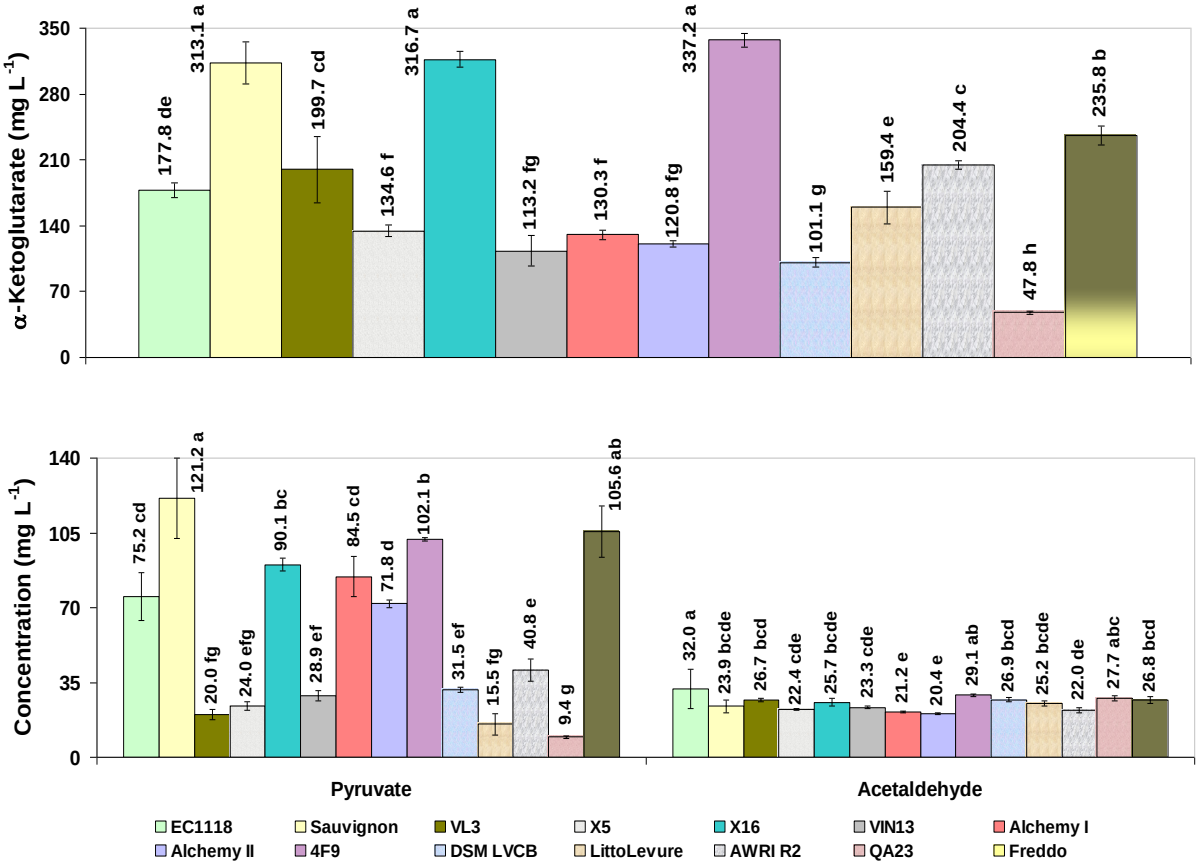


Figure 4-30 Concentration of SO_2-binding compounds in finished YPF wines produced by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound according to the DMRT test.

SO_2-binding compounds: The results show that fourteen *Saccharomyces* yeast strains produced different concentrations of these compounds in final YPF wines (**Figure 4-30**). The Sauvignon, 4F9 and X16 strain appeared to be the most α-ketoglutarate producers in YPF wines (313.3 ± 22.8, 337.2 ± 7.5 and 316.7 ± 8.5 mg L^{-1}, respectively). The first strain was also the greatest pyruvate producer (121.2 ± 18.7 mg L^{-1}) as well strain Freddo (105.6 ± 11.9 mg L^{-1}). While, strain QA23 was the least producer of both keto acids (47.8 ± 1.8 and 9.4 ± 0.7 mg L^{-1}, respectively), on the contrary, it produced a great amount of acetaldehyde (27.7 ± 1.2 mg L^{-1}) in the YPF wine. Nevertheless, the EC1118 and 4F9 strains produced the highest concentrations of acetaldehyde (32.0 ± 9.3 and 29.1 ± 0.5 mg L^{-1}, respectively), whereas this compound was least present in the YPF wines from strains Alchemy I and II.

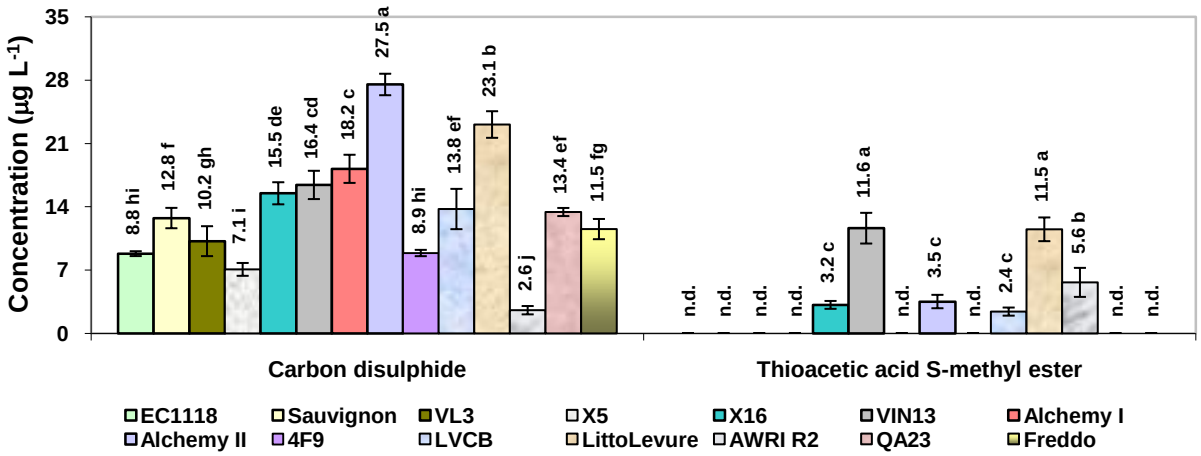


Figure 4-31 Concentration of carbon disulphide and thioacetic acid S-methylester found in finished YPF wines produced by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound according to the DMRT test.

n.d. denotes not detected.

Sulphur containing compounds: **Figure 4-31** shows the significant differences of these compounds produced by the fourteen yeast strains. The Alchemy II and LittoLevure strains were the greatest carbon disulphide producers (27.5 ± 1.2 and 23.1 ± 1.5 µg L^{-1}, respectively), while strain AWRI R2 was the lowest producer. The highest amounts of thioacetic acid S-methylester were produced by strains VIN13 and LittoLevure (11.6 ± 1.7 and 11.5 ± 1.3 µg L^{-1}, respectively), followed by AWRI R2, X16, Alchemy II and LVCB (5.6 ± 1.6, 3.2 ± 0.4, 3.5 ± 0.7 and 2.4 ± 0.4 µg L^{-1}, respectively), while other yeast strains did not produce MeSAc. In addition, all YPF wines did not reveal the other S-compounds, H_2S, DMS, DMDS, ethanethiol, methanethiol and thioacetic acid S-ethylester.

Higher alcohols: **Figure 4-32** shows that concentrations in YPF wines significantly differed depending on the yeast strains used. Strains X16 and AWRI R2 were the highest producers of 2-methyl propanol (154.5 ± 7.9 and 143.7 ± 16.3 mg L^{-1}, respectively), followed by strain Sauvignon (131.5 ± 1.9 mg L^{-1}). Strain LittoLevure developed the greatest amounts of 3-methyl butanol, 2-methyl butanol and 2-phenyl ethanol (206.1 ± 12.4, 38.0 ± 3.2 and 40.2 ± 6.1 mg L^{-1}, respectively). 2-Phenyl ethanol was also most present in the QA23 wine (39.5 ± 2.2 mg L^{-1}) but least amount of 2-methyl propanol (24.8 ± 0.8 mg L^{-1}) was detected in this wine. The 3-methyl and 2-methyl butanol were also most evident in the AWRI R2 and VIN13 wines (185.0 ± 16.0, 31.6 ± 3.2 mg L^{-1} and 170.7 ± 15.3, 33.6 ± 3.6 mg L^{-1}, respectively).

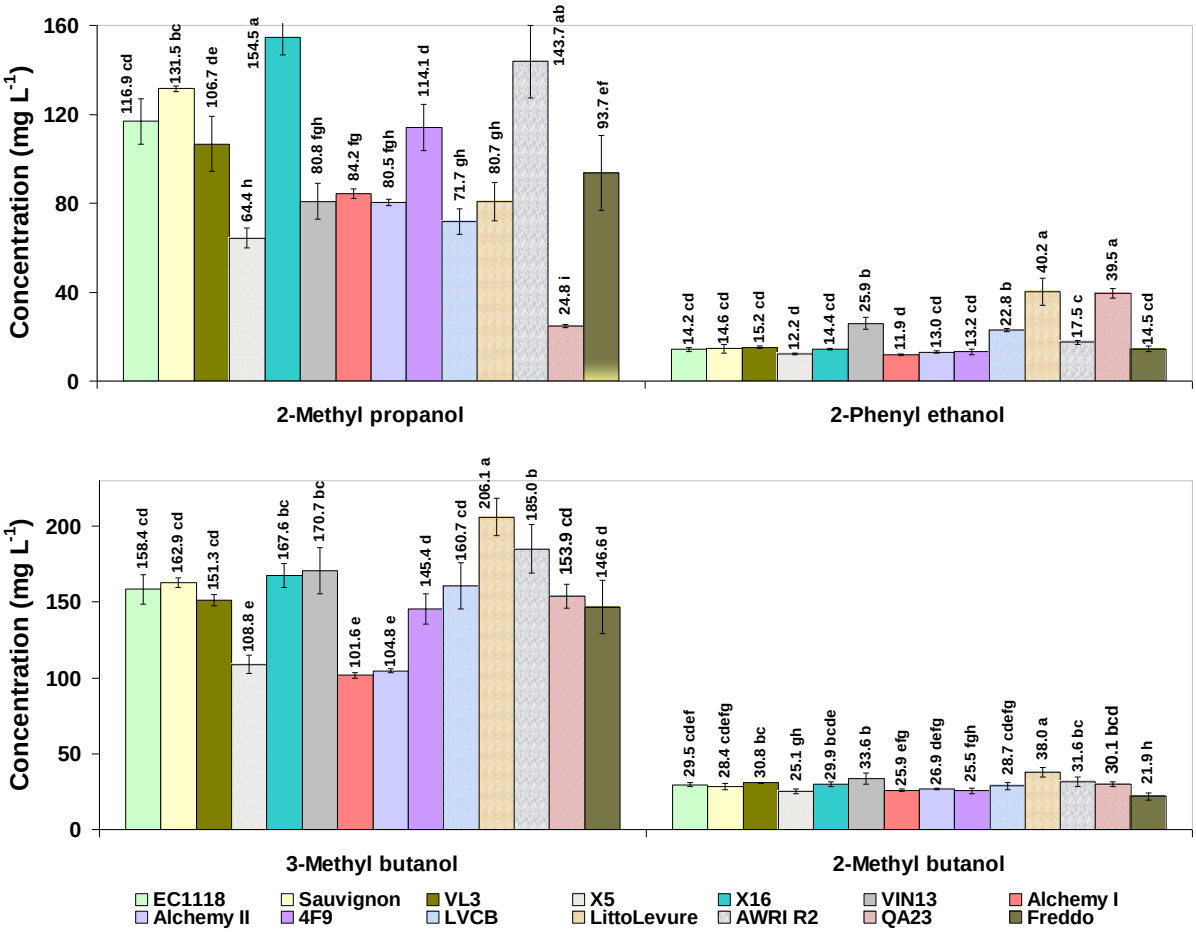


Figure 4-32 Concentration of higher alcohols found in finished YPF wines developed by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains of each compound according to the DMRT test.

Acetic acid esters: The results in **Figure 4-33** show that concentrations of acetic acid ethyl ester in YPF wine were out of the calibration standard value, therefore they were present as relative peak area. The production of acetic acid ethyl ester was slightly different among yeast strains, strains EC1118, X5, VIN13 and Alchemy II tented to be the highest producers (relative peak area ranging from 8.8 $\pm$ 0.0 to 8.9 $\pm$ 0.5), while strains 4F9 and Freddo were the lowest producers. The LittoLevure strain was the most predominant producer of the other acetic acid esters, acetic acid 3- and 2-methylbutyl ester, acetic acid 2-phenylethyl ester and acetic acid hexyl ester (1729.0 $\pm$ 188.9, 72.9 $\pm$ 17.0, 236.7 $\pm$ 30.6 and 14.1 $\pm$ 1.4 $\mu g\ L^{-1}$, respectively). The QA23 and VIN13 followed a similar pattern of these ester productions, however in slightly lower amounts, except for the latter ester that they also produced in the highest amounts (14.3 $\pm$ 0.6 and 13.2 $\pm$ 0.7 $\mu g\ L^{-1}$, respectively).

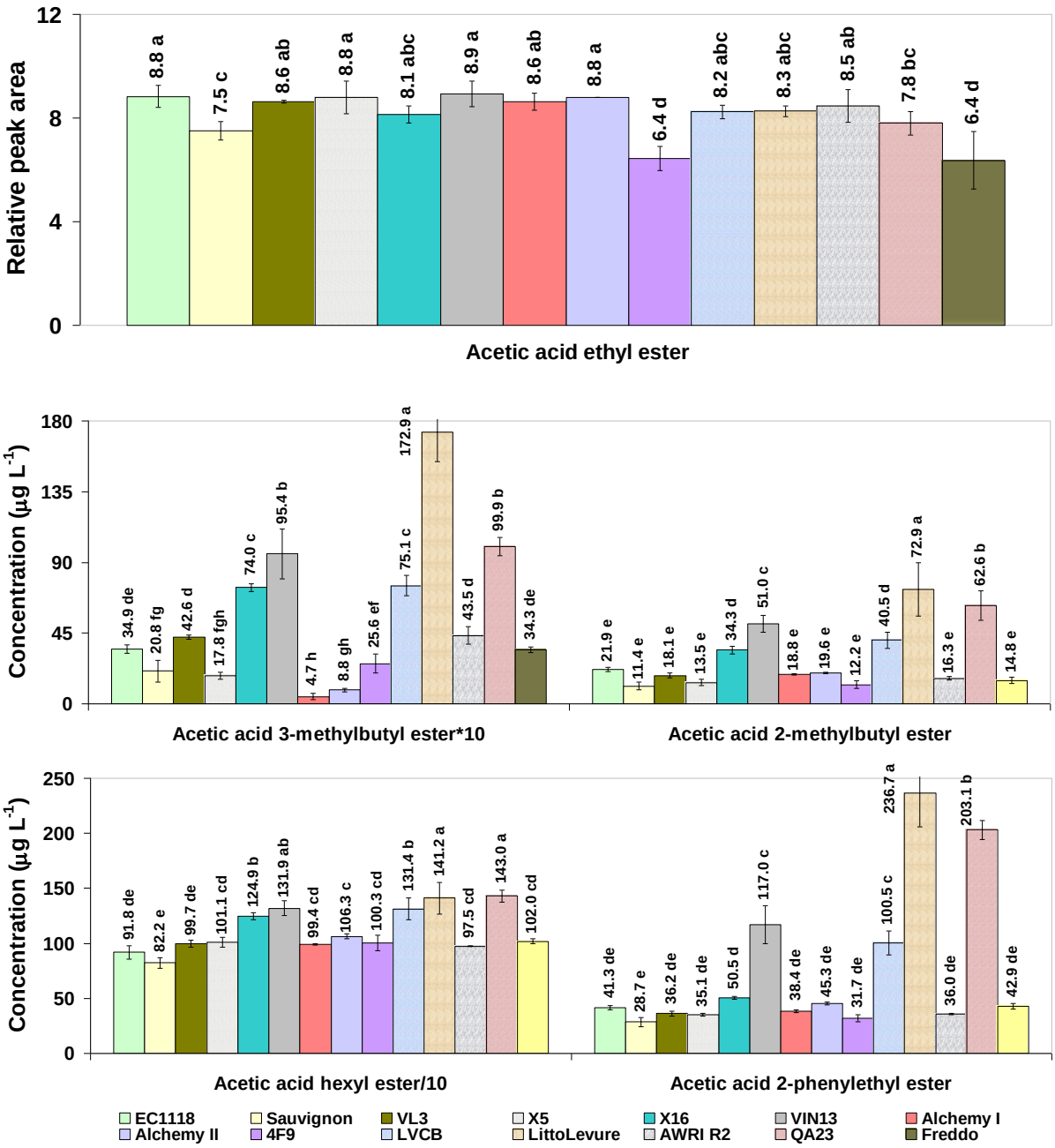


Figure 4-33 Concentration of acetic acid esters found in finished YPF wines developed by 14 different yeast strains (*10 = concentration of compound times 10, whereas /10 = concentration of compound divides by 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound according to the DMRT test.

Relative peak area = (peak area of sample)/(Peak area of internal standard)

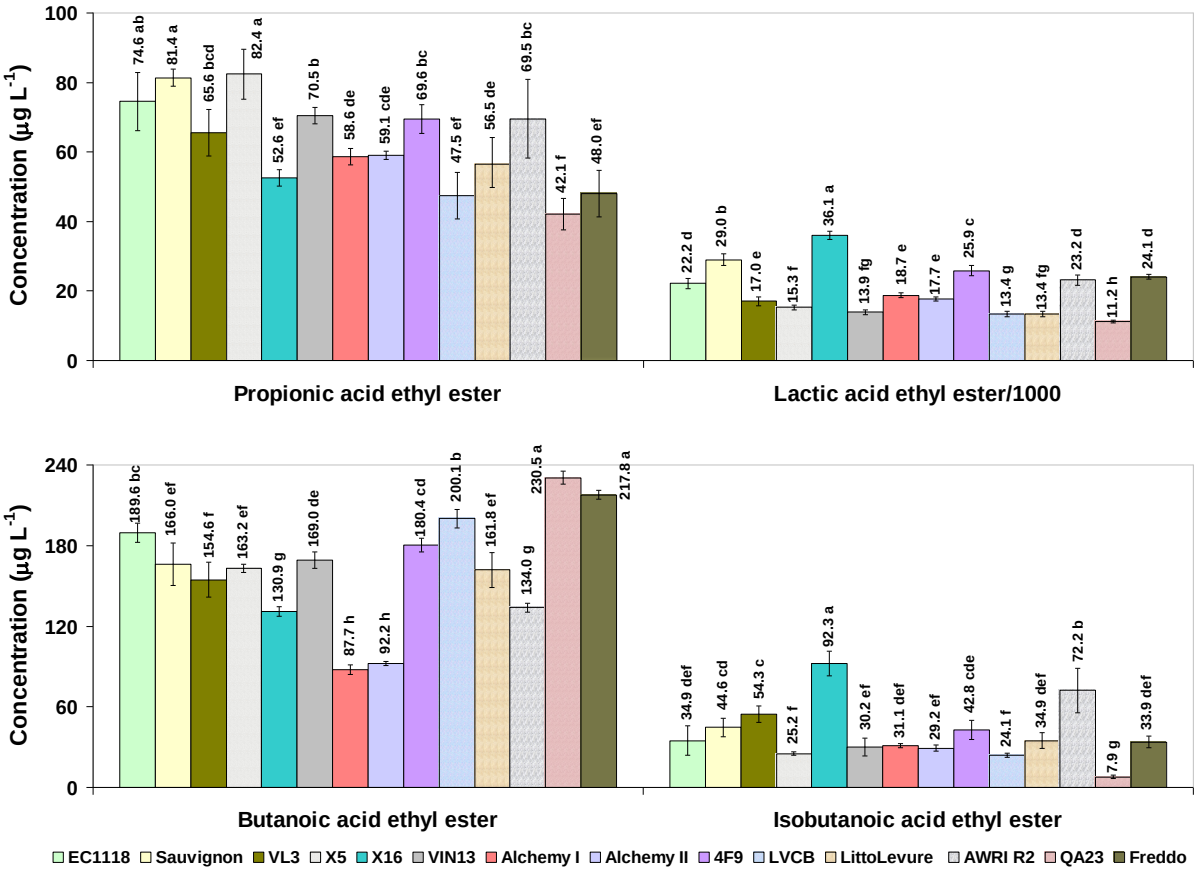


Figure 4-34 Concentration of ethyl esters found in finished YPF wines developed by 14 different yeast strains (/1000 = concentration of compound divides by 1000)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound according to the DMRT test.

Ethyl esters: **Figure 4-34** shows that strains Sauvignon, X5 and EC1118 were the greatest producers of propionic acid ethyl ester (81.4 ± 2.5, 82.4 ± 7.2 and 74.6 ± 8.4 µg L^{-1}, respectively), followed by strain VIN13 (70.5 ± 2.3 µg L^{-1}). The X16 was the highest producer of ethyl esters of lactic acid and isobutanoic acid (36.1 ± 1.2 mg L^{-1} and 92.3 ± 9.2 µg L^{-1}, respectively), followed by strain Sauvignon in respect to the former ester (29.0 ± 1.6 mg L^{-1}), and strain AWRI R2 regarding the latter ester (72.2 ± 16.4 µg L^{-1}). For butanoic acid ethyl ester, the QA23 and Freddo strains were the most predominant producers (230.5 ± 4.8 and 217.8 ± 3.3 µg L^{-1}, respectively) and were followed by strains LVCB and EC1118 (200.1 ± 6.8 and 189.6 ± 7.2 µg L^{-1}, respectively), while the Alchemy I and II were the lowest producers (87.7 ± 3.6 and 92.2 ± 1.4 µg L^{-1}, respectively). Of all ethyl esters, strain QA23 produced the least concentration of ethyl esters, except for butanoic acid ethyl ester.

Monoterpenes: The results show that fourteen yeast strains released significantly different linalool concentrations, but they had similar ability to liberate α-terpineol ranging from 12.1 ± 0.2 to 13.2 ± 0.6 μg L^{-1} (**Figure 4-35**). However, *cis*-linalool oxide and *trans*-linalool oxide were not detected in all YPF wines. Regarding the linalool, strain QA23 was the most releasing strain (11.7 ± 0.8 μg L^{-1}), followed by strains Alchemy II and I and 4F9 (11.4 ± 1.8, 10.0 ± 1.0 and 10.1 ± 0.9 μg L^{-1}, respectively), whereas strains X16 and VL3 were the least releasers.

Medium-chain fatty acids and their ethyl esters: The formation of these compounds were significantly different among the fourteen commercial yeast strains used (**Table 4-18** and **Figure 4-36**). The QA23 was the greatest producer of all MCFAs; hexanoic, octanoic and decanoic acid (6.2 ± 0.0, 7.3 ± 0.6 and 1.6 ± 0.1 mg L^{-1}, respectively), and their ethyl esters; HAEtE, OAEtE and DAEtE (758.6 ± 48.7, 1111.6 ± 60.7 and 387.0 ± 32.8 μg L^{-1}, respectively). High concentrations of these compounds were also produced by strains LVCB (5.7 ± 0.0, 6.3 ± 0.0, 1.3 ± 0.1 mg L^{-1} and 564.0 ± 5.8, 1004.4 ± 19.4, 338.9 ± 9.4 μg L^{-1}, respectively) and Freddo (5.1 ± 0.6, 5.3 ± 0.6, 1.1 ± 0.1 mg L^{-1} and 485.2 ± 4.9, 816.5 ± 18.8, 250.9 ± 16.9 μg L^{-1}, respectively). On the contrary, strain X16 was the least MCFA and ethyl ester producer.

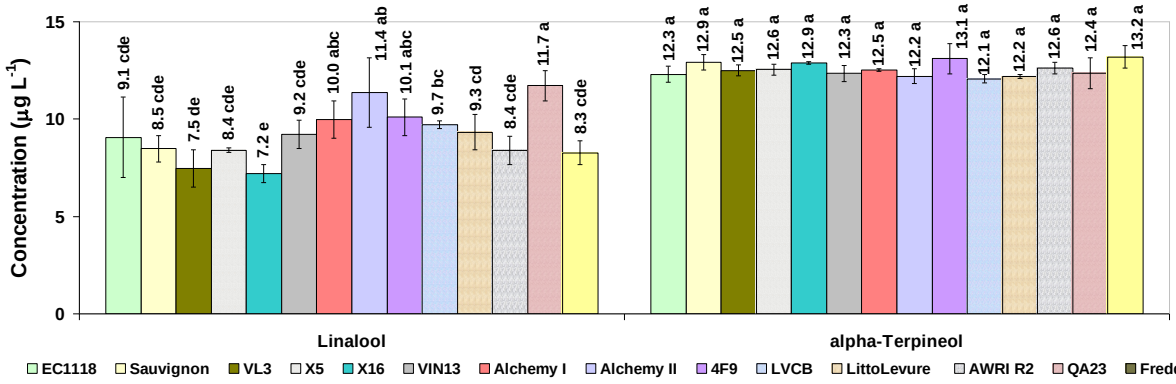


Figure 4-35 Concentration of linalool and α-terpineol found in finished YPF wines developed by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound, whereas those displaying the same letters are not significantly different ($p>0.05$) according to the DMRT test.

Table 4-18 Concentration of fatty acids found in finished YPF wines developed by 14 yeast strains

Yeast	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})
EC1118	4.2 ± 0.0 d	3.9 ± 0.0 d	0.6 ± 0.1 i
Sauvignon	4.2 ± 0.0 d	4.0 ± 0.0 d	0.6 ± 0.1 hi
VL3	4.4 ± 0.6 cd	4.3 ± 0.0 d	0.8 ± 0.1 efgh
X5	4.5 ± 0.6 cd	4.7 ± 0.6 cd	0.9 ± 0.0 cdef
X16	3.4 ± 0.0 e	2.6 ± 0.6 e	0.3 ± 0.1 j
VIN13	4.2 ± 0.0 cd	4.3 ± 0.0 d	0.7 ± 0.1 fghi
Alchemy I	4.1 ± 0.0 cd	4.2 ± 0.0 d	0.8 ± 0.1 defg
Alchemy II	4.5 ± 0.6 cd	4.6 ± 0.6 cd	1.0 ± 0.1 c
4F9	4.7 ± 0.0 b	4.9 ± 0.0 c	0.9 ± 0.1 cde
LVCB	5.7 ± 0.0 a	6.3 ± 0.0 b	1.3 ± 0.1 b
LittoLevure	4.5 ± 0.6 bc	4.3 ± 0.6 cd	0.7 ± 0.2 ghi
AWRI R2	4.1 ± 0.0 d	3.7 ± 0.0 d	0.6 ± 0.1 hi
QA23	6.2 ± 0.0 a	7.3 ± 0.6 a	1.6 ± 0.1 a
Freddo	5.1 ± 0.6 b	5.3 ± 0.6 c	1.1 ± 0.1 c

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) according to the DMRT test.

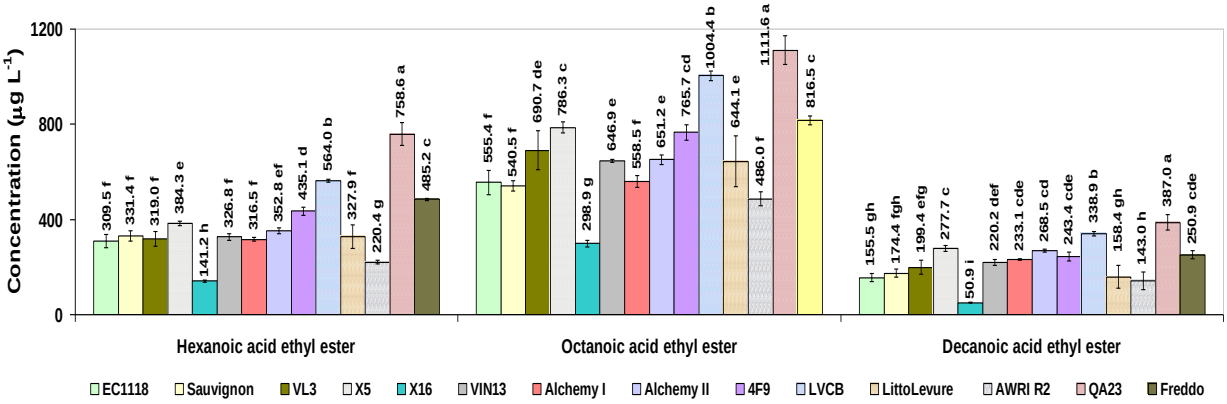


Figure 4-36 Concentration of fatty acid ethyl esters found in finished YPF wines developed by 14 different yeast strains

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between yeast strains of each compound according to the DMRT test.

4.3.2 Effect of three commercial *Saccharomyces* strains and five different commercial nutrient sources on growth kinetics, fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

The fermentation trial was performed in yellow passion fruit (YPF) juice in 2008. Three commercial yeast strains; EC1118, Sauvignon and X5, and five commercial nutrient sources, DAP, Vitamon Combi (VCombi), Vitamon Ultra (VUltra), Fermaid E blanc (Fermaid E) and OptiWhite at 0.4 g L^{-1} were used in the fermentation YPF trial. The fermentation kinetics and some non-volatile and volatile compounds in final YPF wines were examined. The two *S. cerevisiae* yeast strains; Sauvignon and X5, were chosen from the basis results obtained from a previous trial of Sauvignon blanc and Scheurebe grape wines as well as the YPF trial. They are considered to have relatively high nitrogen demands and their fermentation performances are considered to be more susceptible to nitrogen deficiency. Additionally, they also tended to produce wines having desirable fermentative characteristics and quality. Whereas, the EC1118 strain was used as a control strain due to its high popularity in wine industry and its tendency to produce less undesirable components.

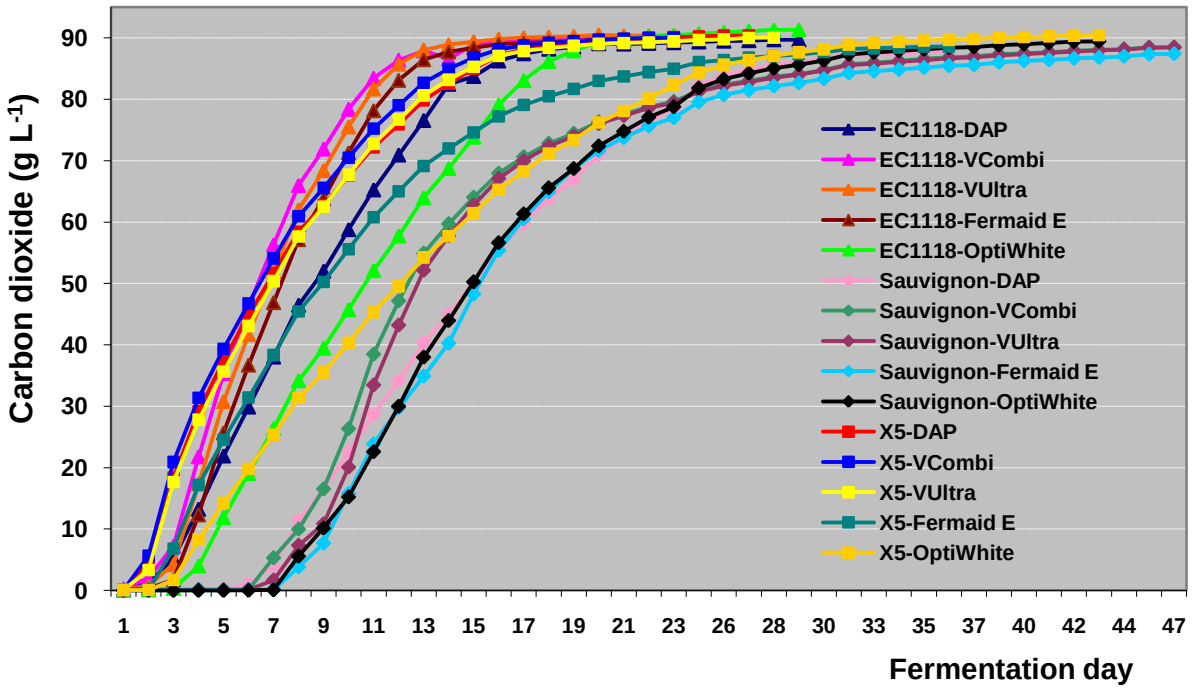


Figure 4-37 Fermentation kinetics of YPF juice fermentations with three different commercial yeast strains and four different nutrient sources

The fermentation kinetics: As shown in **Figure 4-37**, the EC1118 strain seemed to have the fastest growth kinetics and time, while strain Sauvignon had the slowest kinetics, however varied depending on the nutrient source. The addition of VCombi, VUltra and Fermaid E enhanced strain EC1118 to have a similarly short lag phase and helped to finish fermentations after 23 days, on the contrary, in the DAP and OptiWhite samples they prolonged the fermentations to finish after 29 days. For strain X5, the fermentation kinetics were relatively more responsive to the nutrient source than strain EC1118. Thus, the duration of fermentation was shortest in the VCombi treatment (23 days), while addition of other nutrients like DAP, VUltra, Fermaid E and OptiWhite, seemed to prolong the fermentation time (27, 28, 36 and 43 days of fermentation, respectively). Nevertheless, the YPF fermentations with both yeast strains completely finished. The Sauvignon strain tended to have inverse behaviour. In all nutrient sources, its fermentations had a long lag phase (5-12 days) and resulted in sluggish fermentation varying from 36 to 47 days. Nonetheless, all YPF fermentations with this strain completely finished as well.

Chemical composition: Three yeast strains produced YPF wines having different amounts of residual sugar in response to the nutrient source (**Table 4-19**). Concentrations of residual sugar were most evident in YPF wines produced by strain Sauvignon with the addition of VUltra, Fermaid E and OptiWhite (1.5 $\pm$ 0.2, 1.6 $\pm$ 0.1 and 1.4 $\pm$ 0.3 g L^{-1}, respectively), followed by with VCombi supplementation (1.1 $\pm$ 0.2 g L^{-1}). Nevertheless, the three yeast strains consumed practically almost the initial sugar concentration provided (201 g L^{-1} as given in **3.9.2**) and resulted in low residual sugar below 2 g L^{-1}, which helped to achieve dryness in final YPF wines. In addition, there was no significant difference in glycerol formation among the treatment combinations ranging from 4.6 $\pm$ 1.1 to 6.2 $\pm$ 0.3 g L^{-1}.

Organic acids: The results demonstrate that the commercial yeast strains and the nutrient sources significantly affected the concentration of organic acids in YPF wines (**Table 4-20**). Strain Sauvignon synthesized the highest amounts of malic acid in the DAP treatment (1.47 $\pm$ 0.24 g L^{-1}) and acetic acid in the Fermaid E and OptiWhite samples (0.47 $\pm$ 0.05 and 0.48 $\pm$ 0.04 g L^{-1}, respectively). Strain Sauvignon synthesized malic acid approximately 64.7 % in the DAP treatment and 39.5 % in the VCombi fermentation, while strain X5 produced 39.5 % malic acid in the Fermaid E treatment. The highest amounts of citric acid were present in YPF wine of strain X5 with the addition of OptiWhite (3.70 $\pm$ 0.03 g L^{-1}) and strain Sauvignon with the DAP treatment (3.67 $\pm$ 0.04 g L^{-1}).

Table 4-19 Chemical composition of finished YPF wines obtained from frozen YPF juices fermented with three different yeast strains and five nutrient sources

Yeast	Nutrient	Residual sugar (g L^{-1})	Glycerol (g L^{-1})
EC1118	DAP	0.2 ± 0.1 ef	4.8 ± 0.1 a
	VCombi	0.4 ± 0.2 def	4.9 ± 0.0 a
	VUltra	0.2 ± 0.1 f	4.9 ± 0.1 a
	Fermaid E	0.5 ± 0.2 cde	4.8 ± 0.2 a
	OptiWhite	0.5 ± 0.2 cdef	4.8 ± 0.1 a
Sauvignon	DAP	0.7 ± 0.2 c	5.9 ± 0.1 a
	VCombi	1.1 ± 0.2 b	6.2 ± 0.3 a
	VUltra	1.5 ± 0.2 a	5.9 ± 0.2 a
	Fermaid E	1.6 ± 0.1 a	6.1 ± 0.1 a
	OptiWhite	1.4 ± 0.3 a	5.4 ± 0.5 a
X5	DAP	0.4 ± 0.1 def	4.9 ± 0.0 a
	VCombi	0.3 ± 0.1 ef	4.8 ± 0.1 a
	VUltra	0.3 ± 0.1 ef	4.7 ± 0.1 a
	Fermaid E	0.5 ± 0.2 cdef	4.7 ± 0.1 a
	OptiWhite	0.6 ± 0.1 cd	4.6 ± 1.1 a

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

SO_2-binding compounds: **Figure 4-38** shows that the commercial yeast strains and the nutrient sources significantly affected the production of these compounds in YPF wines. In the DAP treatment, the greatest concentrations of α-ketoglutarate were formed by strain Sauvignon (338.1 ± 22.9 mg L^{-1}), followed by strain EC1118 (150.2 ± 31.5 mg L^{-1}) and that of pyruvate by these two strains (62.0 ± 14.4 and 61.5 ± 6.8 mg L^{-1}, respectively). The X5 seemed to be the lowest producer of both keto acids with less response to nutrient sources, although the production slightly increased in the OptiWhite sample. Nevertheless, the addition of VCombi, VUltra and Fermaid E significantly diminished the formation of these keto acids. On the other hand, these three nutrient sources stimulated the highest acetaldehyde formation in YPF wines by the EC1118 strain (31.6 ± 0.2, 29.9 ± 0.9 and 30.3 ± 0.5 mg L^{-1}, respectively). This compound was also most evident in the YPF fermentation with strain X5 in the presence of VCombi (29.8 ± 1.9 mg L^{-1}).

Table 4-20 Concentration of organic acids found in finished YPF wines produced by three different yeast strains with five nutrient sources

Yeasts	Nutrients	Malic acid (g L^{-1})	Acetic acid (g L^{-1})	Citric acid (g L^{-1})
EC1118	DAP	0.66 ± 0.01 cd	0.27 ± 0.01 def	3.53 ± 0.01 fgh
	VCombi	0.55 ± 0.01 d	0.28 ± 0.03 de	3.54 ± 0.02 efgh
	VUltra	0.58 ± 0.01 d	0.29 ± 0.01 cd	3.55 ± 0.02 defgh
	Fermaid E	0.60 ± 0.01 d	0.34 ± 0.01 bc	3.58 ± 0.05 cdef
	OptiWhite	0.63 ± 0.01 cd	0.38 ± 0.01 b	3.57 ± 0.01 def
Sauvignon	DAP	1.47 ± 0.24 a	0.35 ± 0.04 b	3.67 ± 0.04 ab
	VCombi	0.89 ± 0.27 b	0.35 ± 0.07 b	3.56 ± 0.04 defg
	VUltra	0.74 ± 0.01 bcd	0.38 ± 0.02 b	3.59 ± 0.01 cde
	Fermaid E	0.72 ± 0.03 bcd	0.47 ± 0.05 a	3.61 ± 0.01 cd
	OptiWhite	0.62 ± 0.04 cd	0.48 ± 0.04 a	3.64 ± 0.02 bc
X5	DAP	0.68 ± 0.03 cd	0.27 ± 0.00 def	3.50 ± 0.00 gh
	VCombi	0.73 ± 0.03 bcd	0.22 ± 0.01 f	3.59 ± 0.02 cdef
	VUltra	0.80 ± 0.04 bc	0.23 ± 0.01 ef	3.49 ± 0.01 h
	Fermaid E	0.86 ± 0.01 b	0.24 ± 0.00 ef	3.60 ± 0.01 cd
	OptiWhite	0.79 ± 0.01 bc	0.34 ± 0.01 bc	3.70 ± 0.03 a

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Sulphur containing compounds: The results in **Figure 4-39** demonstrate that three commercial yeast strains significantly produced different compounds in response to the nutrient source. Addition of DAP promoted the greatest production of H_2S and CS_2 by strain EC1118 (7.2 ± 1.5 and 19.1 ± 2.8 μg L^{-1}, respectively), whereas addition of VCombi and VUltra resulted in the highest formation of methanethiol (7.3 ± 1.0 and 7.4 ± 1.0 μg L^{-1}, respectively). However, methanethiol was either not detected or present in trace quantities in the other YPF wine treatments. Strain Sauvignon seemed to be the most predominant CS_2 producer with less response to the nutrient source (15.0 ± 1.6 to 21.8 ± 3.6 μg L^{-1}) and also developed a high level of H_2S in the presence of DAP (5.3 ± 1.2 μg L^{-1}). Under the condition in this trial, the other sulphur compounds, ethanethiol, dimethyl sulphide, dimethyl disulphide, thioacetic acid S-methylester, thioacetic acid S-ethylester, diethyl disulphide and dimethyl trisulphide were not detected in all YPF wines.

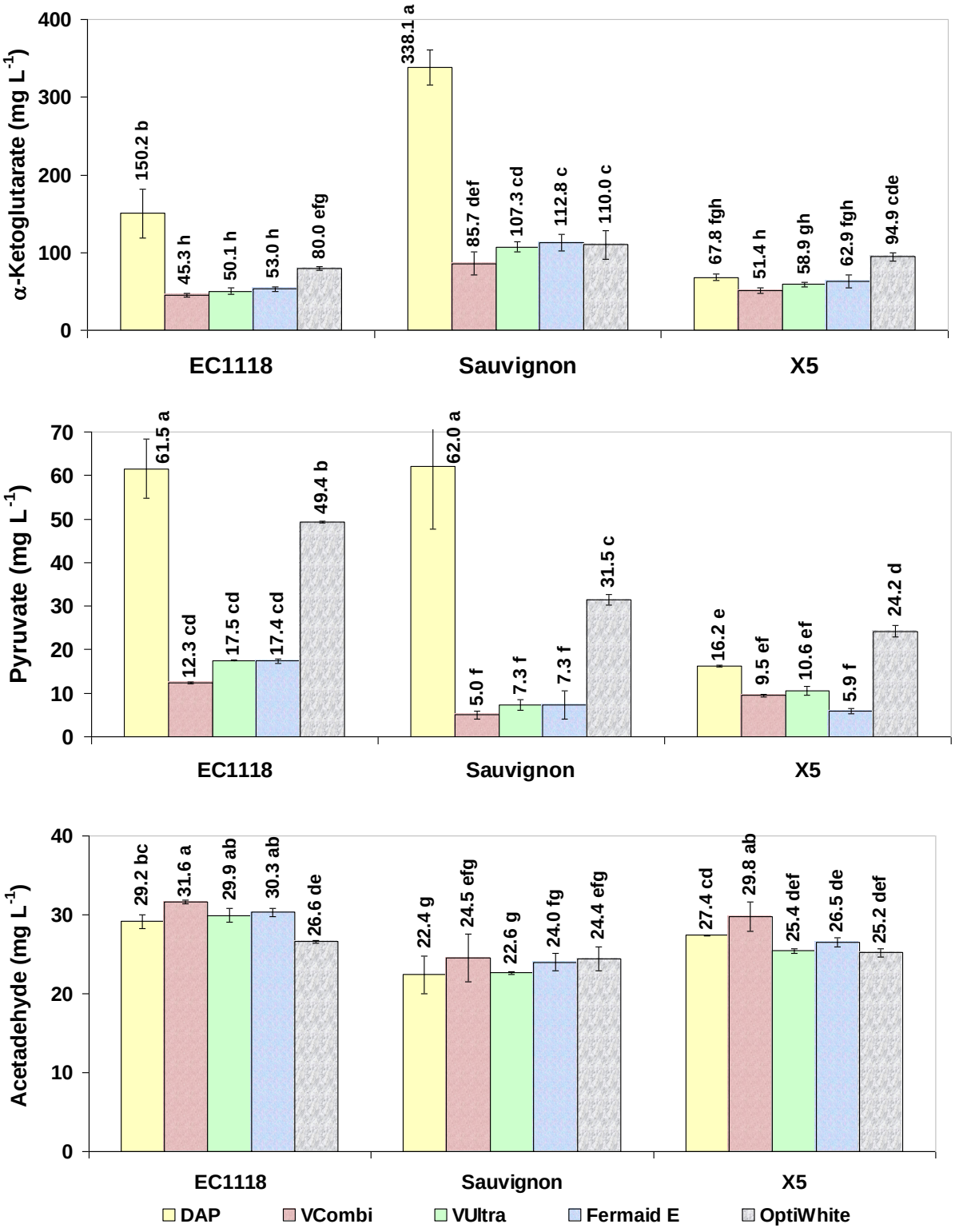


Figure 4-38 Concentration of SO_2-binding compounds found in finished YPF wines produced by three different yeast strains with five nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

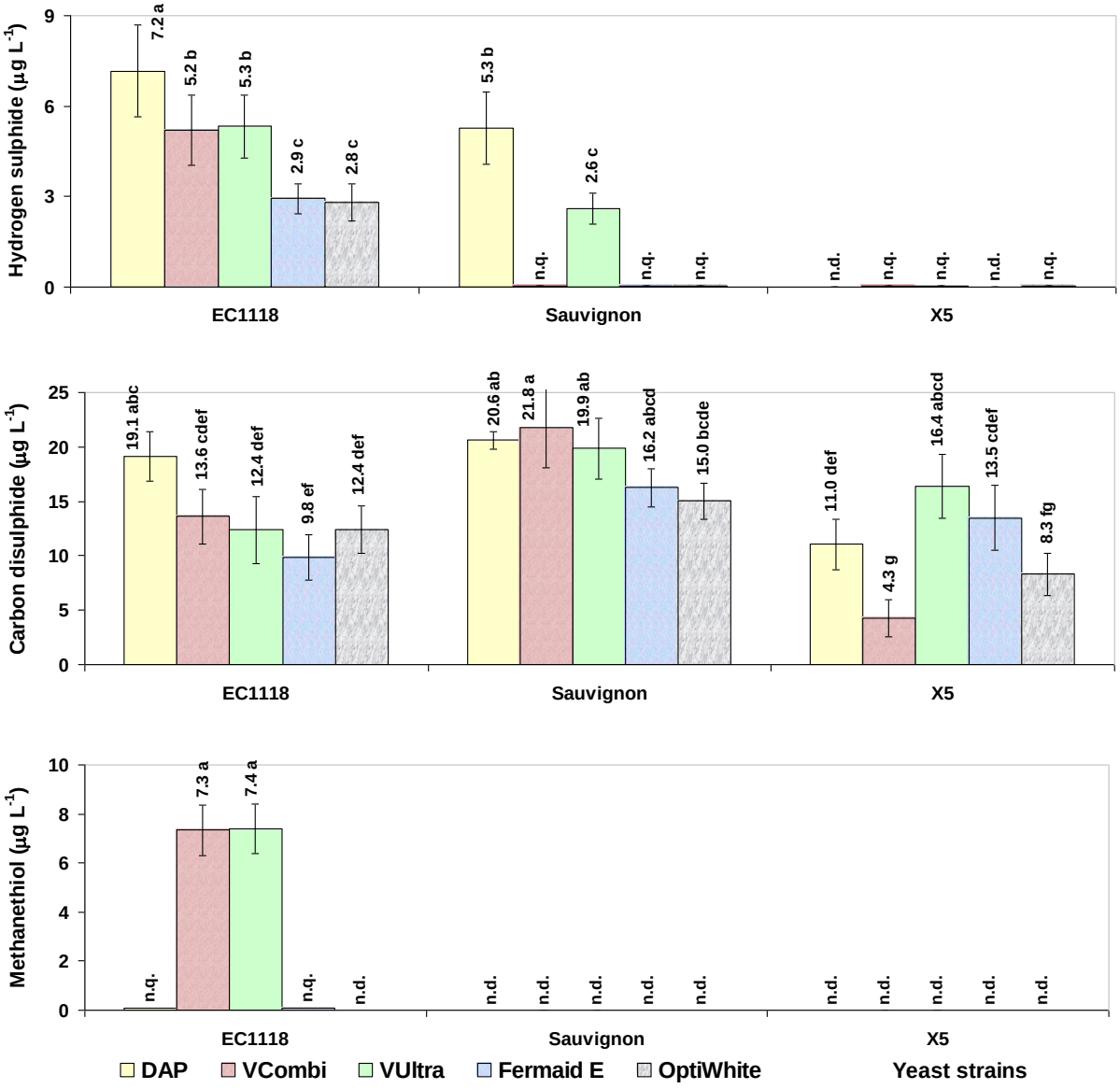


Figure 4-39 Concentration of carbon disulphide and thioacetic acid S-methylester found in finished YPF wines produced by three different yeast strains with five nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

n.d. denotes not detected.

n.q. denotes not quantified or traced.

Higher alcohols: **Figure 4-40** demonstrates that the Sauvignon strain was the predominant producer of higher alcohols, although its production varied depending on the nutrient source. It developed the greatest amounts of 2-methyl butanol and 2-phenyl ethanol in the VCombi, VUltra and Fermaid E samples (47.5 ± 4.9, 50.0 ± 2.9, 47.5 ± 3.6 mg L^{-1} and 52.7 ± 6.1, 55.0 ± 0.3, 56.0 ± 1.0 mg L^{-1}, respectively). The highest level of 2-methyl propanol was also produced by strain Sauvignon in the presence of DAP (143.6 ± 23.1 mg L^{-1}), while 3-methyl butanol was most evident in the DAP, VCombi, VUltra and Fermaid E treatments (181.5 ± 20.3, 187.3 ± 8.0, 197.7 ± 10.0 and 195.1 ± 11.0 mg L^{-1}, respectively). Addition of DAP significantly reduced the formation of 2-methyl butanol and 2-phenyl ethanol for all yeast strains, while VCombi, VUltra and Fermaid E decreased the formation of 2-methyl propanol.

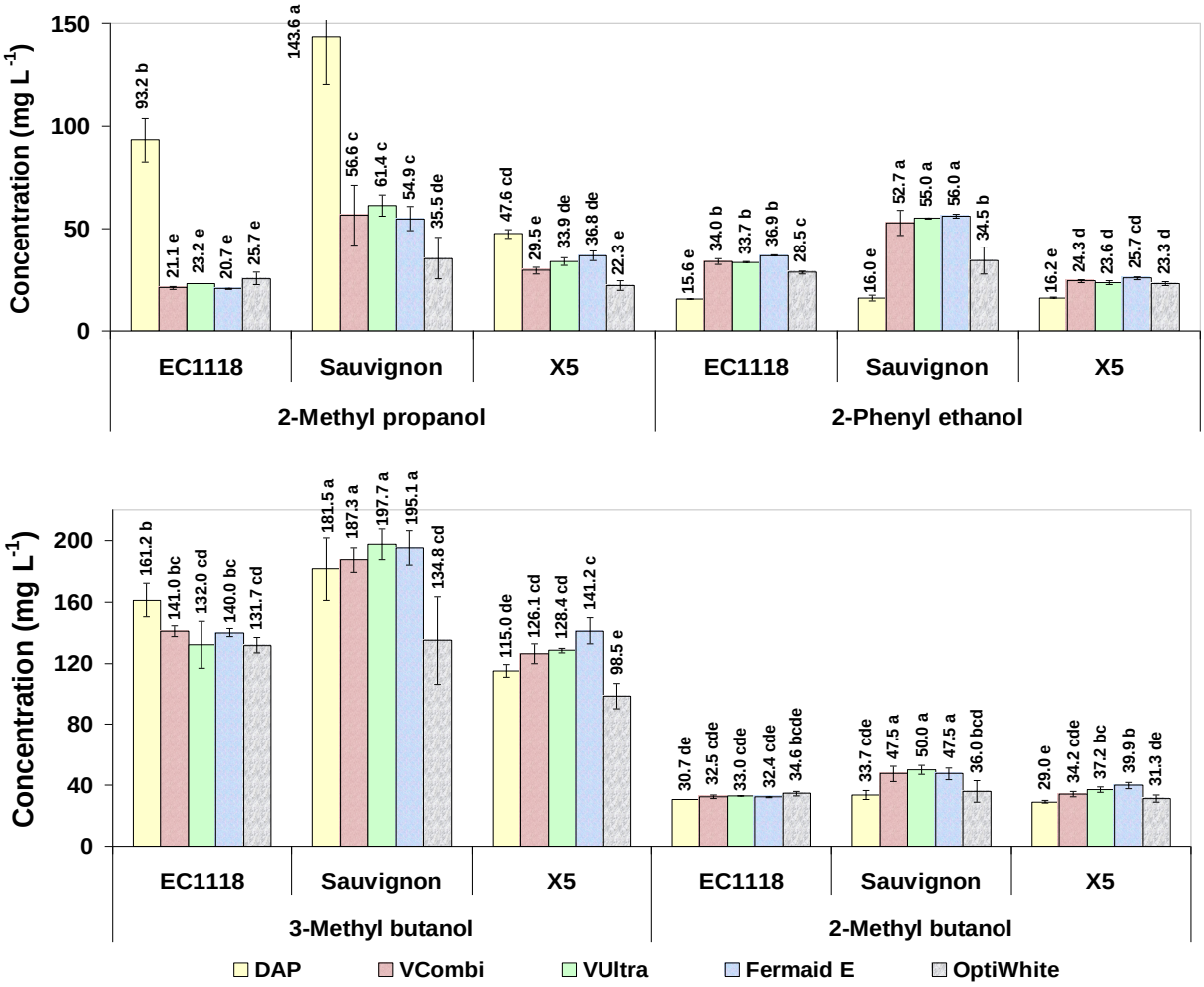


Figure 4-40 Concentration of higher alcohols found in finished YPF wines developed by three different yeast strains with five nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

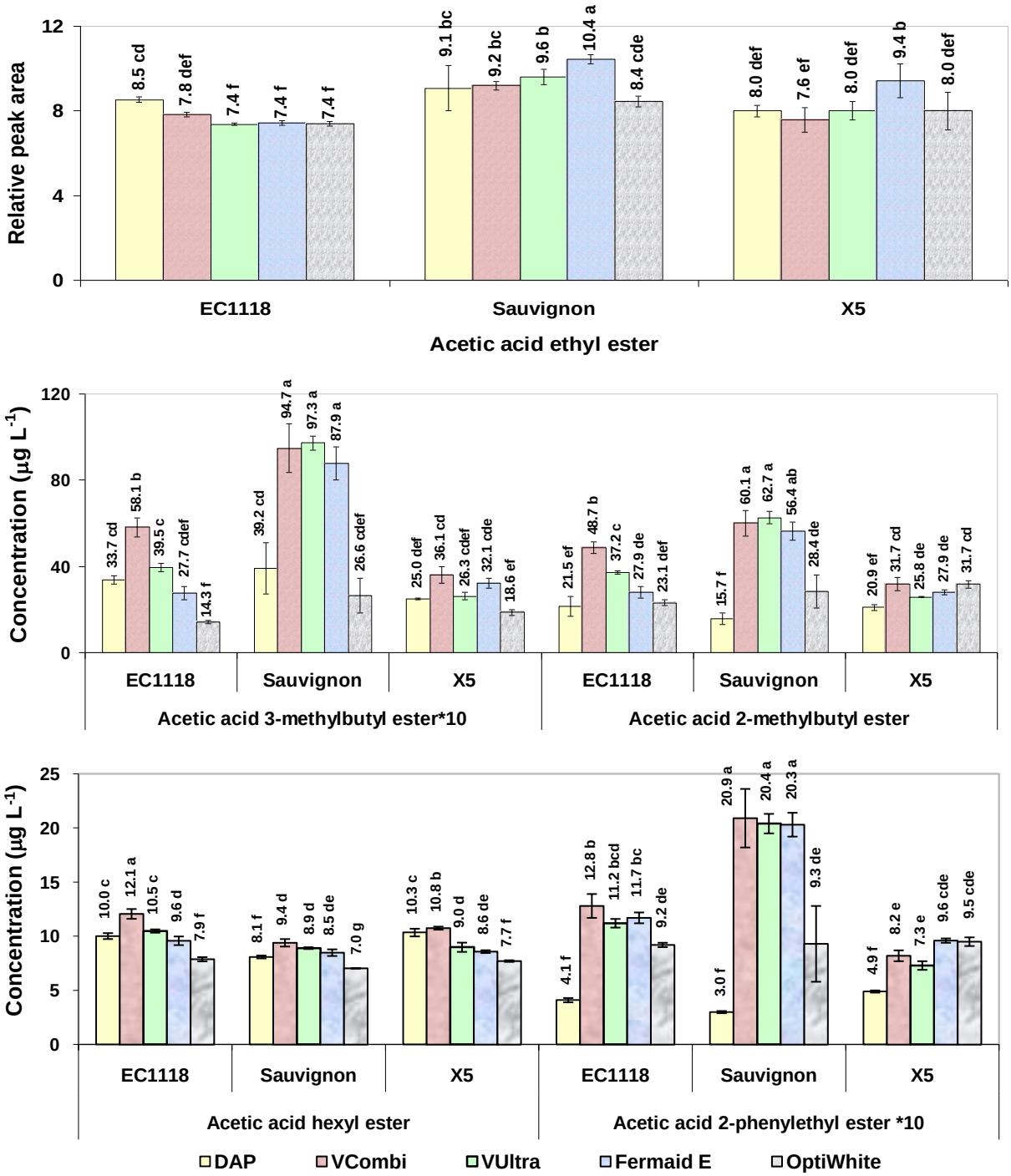


Figure 4-41 Concentration of acetic acid esters found in finished YPF wines developed by three different yeast strains with five nutrient sources (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

Relative peak area = (peak area of sample)/(Peak area of internal standard)

Acetic acid esters: **Figure 4-41** shows that the Sauvignon strain tended to be the greatest acetic acid ester producer, excluding the acetic acid hexyl ester, however this depended on the nutrient source. Concentrations of acetic acid ethyl ester in YPF wine were out of the quantification limit, they were therefore present as relative peak area. Addition of Fermaid E promoted the highest formation of acetic acid ethyl ester by strain Sauvignon (10.4 $\pm$ 0.2 relative peak area) and followed by strain X5. Strain Sauvignon with the addition of VCombi, VUltra and Fermaid E developed the greatest amounts of acetic acid 3-methylbutyl ester (947.2 $\pm$ 113.1, 972.8 $\pm$ 32.4, 878.9 $\pm$ 76.3 $\mu g\ L^{-1}$, respectively), acetic acid 2-methylbutyl ester (60.1 $\pm$ 6.0, 62.7 $\pm$ 2.9, 56.4 $\pm$ 4.2 $\mu g\ L^{-1}$, respectively) and acetic acid 2-phenyl ethyl ester (208.9 $\pm$ 27.5, 203.8 $\pm$ 8.7, 203.3 $\pm$ 11.3 $\mu g\ L^{-1}$, respectively). On the contrary, DAP addition significantly reduced the levels of these compounds for all yeast strains. Acetic acid hexyl ester was most evident in the EC1118 strain with VCombi treatment, followed by the X5 strain with the same nutrient (12.1 $\pm$ 0.5 and 10.8 $\pm$ 0.1 $\mu g\ L^{-1}$, respectively).

Ethyl and diethyl esters: As shown in **Table 4-21**, SAdiEtE was detected only in trace quantities in all YPF wines (data not shown). The X5 strain was the highest PrEtE producer when either Fermaid E or OptiWhite was added to the YPF juices (151.0 $\pm$ 14.7 and 161.9 $\pm$ 12.0 $\mu g\ L^{-1}$, respectively). High production of BuEtE was produced by strain EC1118 in the VCombi sample (210.4 $\pm$ 16.1 $\mu g\ L^{-1}$), followed by the DAP and VUltra treatments (181.2 $\pm$ 11.0 and 180.4 $\pm$ 5.1 $\mu g\ L^{-1}$, respectively), while strain X5 also developed high amount of this ester in the VCombi sample (176.6 $\pm$ 8.7 $\mu g\ L^{-1}$). Addition of DAP promoted the Sauvignon strain to develop the greatest amounts of iBuEtE and LAEtE (56.8 $\pm$ 9.9 and 25.8 $\pm$ 0.3 $\mu g\ L^{-1}$, respectively). Strain EC1118 seemed to follow the similar pattern for these ester productions in response to DAP addition (23.5 $\pm$ 1.0 and 14.9 $\pm$ 1.6 $\mu g\ L^{-1}$, respectively).

Medium-chain fatty acids and ethyl esters: As shown in **Table 4-22**, addition of VCombi and VUltra promoted strain EC1118 to develop the greatest amounts of hexanoic, octanoic and decanoic acids (5.3 $\pm$ 0.3 and 4.9 $\pm$ 0.3 mg L^{-1}, 6.0 $\pm$ 0.5 and 5.5 $\pm$ 0.2 mg L^{-1}, and 1.2 $\pm$ 0.2 and 1.1 $\pm$ 0.0 mg L^{-1}, respectively). Hexanoic acid was also most evident in the EC1118 wine with Fermaid E addition. Strain X5 also produced the highest amounts of all MCFAs in the VCombi treatment (5.1 $\pm$ 0.1, 5.8 $\pm$ 0.2 and 1.2 $\pm$ 0.0 mg L^{-1}, respectively), while hexanoic and octanoic acid were most evident in the DAP sample (4.9 $\pm$ 0.1 and 5.4 $\pm$ 0.2 mg L^{-1}, respectively). It also developed the greatest amounts of OAEtE and DAEtE in the VCombi (954.3 $\pm$ 21.2 and 317.5 $\pm$ 28.4 $\mu g\ L^{-1}$, respectively) and the DAP samples (873.3 $\pm$ 5.7 and 306.0 $\pm$ 5.7 $\mu g\ L^{-1}$, respectively). Addition of VCombi promoted the highest productions of all MCFA ethyl esters by strain EC1118 as well.

Table 4-21 Concentration of ethyl esters found in finished YPF wines produced by three different yeast strains with five nutrient sources

Yeasts	Nutrients	PrEtE ($\mu g\ L^{-1}$)	BuEtE ($\mu g\ L^{-1}$)	iBuEtE ($\mu g\ L^{-1}$)	LAEtE ($mg\ L^{-1}$)
EC1118	DAP	70.7 ± 1.5 ef	181.2 ± 11.0 b	23.5 ± 1.0 bc	14.9 ± 1.6 b
	VCombi	58.9 ± 3.7 f	210.4 ± 16.1 a	7.0 ± 2.2 gh	8.8 ± 0.1 fg
	VUltra	65.2 ± 1.0 ef	180.4 ± 5.1 b	5.9 ± 0.5 h	8.7 ± 0.1 fg
	Fermaid E	68.7 ± 0.9 ef	154.4 ± 6.4 cd	7.6 ± 0.4 gh	8.5 ± 0.1 fg
	OptiWhite	100.8 ± 3.4 c	71.1 ± 2.1 h	11.1 ± 0.2 efgh	8.3 ± 0.2 g
Sauvignon	DAP	70.4 ± 22.6 ef	120.8 ± 17.2 fg	56.8 ± 9.9 a	25.8 ± 0.3 a
	VCombi	67.7 ± 1.7 ef	150.2 ± 15.1 d	22.8 ± 6.2 bc	10.8 ± 0.2 c
	VUltra	76.1 ± 1.5 e	121.4 ± 5.2 fg	22.6 ± 0.4 bc	10.8 ± 0.2 c
	Fermaid E	73.8 ± 3.4 ef	106.2 ± 5.6 g	26.4 ± 2.4 b	10.2 ± 0.4 cd
	OptiWhite	77.4 ± 2.5 de	50.4 ± 1.1 i	22.9 ± 5.2 bc	9.2 ± 0.2 fg
X5	DAP	80.8 ± 1.2 de	167.4 ± 3.9 bc	14.6 ± 0.7 def	10.0 ± 0.2 de
	VCombi	90.7 ± 4.1 cd	176.6 ± 8.7 b	9.3 ± 0.3 fgh	8.7 ± 0.1 efg
	VUltra	121.4 ± 4.7 b	141.7 ± 2.2 de	12.4 ± 1.1 efg	9.1 ± 0.1 efg
	Fermaid E	151.0 ± 14.7 a	133.3 ± 11.3 ef	16.7 ± 2.0 cde	9.5 ± 0.1 def
	OptiWhite	161.9 ± 12.0 a	57.2 ± 1.5 hi	19.5 ± 1.8 cd	8.0 ± 0.1 g

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Monoterpenes: Concentrations of monoterpenes were significantly different depending on the yeast strain rather than on the nutrient source (**Figure 4-42**). The Sauvignon strain seemed to be the most releasing strain of α-terpineol (12.2 ± 0.3 to 13.1 ± 0.1 μg L^{-1}) and linalool (9.9 ± 1.4 to 11.7 ± 1.9 μg L^{-1}), although linalool was decreased in the DAP treatment (7.2 ± 0.9 μg L^{-1}). High concentrations of these compounds were also released by strain X5 in the Fermaid E (12.8 ± 0.2 and 9.8 ± 0.3 μg L^{-1}, respectively) and OptiWhite treatment (12.6 ± 0.7 and 10.8 ± 1.1 μg L^{-1}, respectively). Other monoterpenes, *trans*-linalool oxide and *cis*-linalool oxide were not detected in all YPF wine treatments.

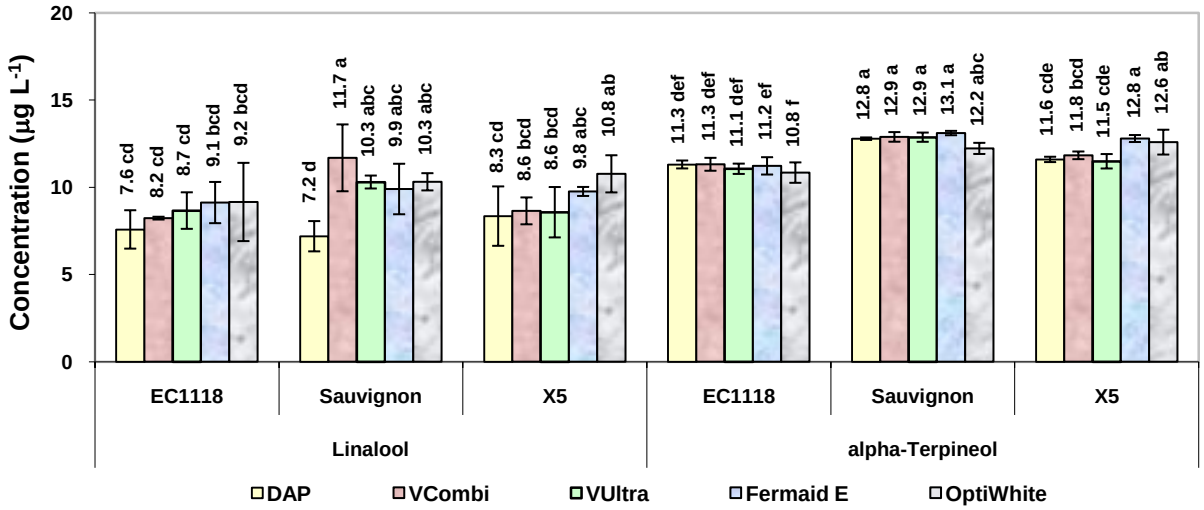


Figure 4-42 Concentration of linalool and α-terpineol found in finished YPF wines developed by three different yeast strains with five nutrient sources

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Table 4-22 Concentration of fatty acids and their ethyl esters found in finished YPF wines produced by three different yeast strains with five nutrient sources

Yeasts	Nutrients	Hexanoic acid (mg L^{-1})	Octanoic acid (mg L^{-1})	Decanoic acid (mg L^{-1})	HAEtE (μg L^{-1})	OAEtE (μg L^{-1})	DAEtE (μg L^{-1})
EC1118	DAP	4.1 ± 0.2 b	3.8 ± 0.2 c	0.5 ± 0.2 ef	377.2 ± 37.8 e	621.4 ± 42.0 d	174.5 ± 13.9 f
	VCombi	5.3 ± 0.3 a	6.0 ± 0.5 a	1.2 ± 0.2 a	634.0 ± 84.4 a	921.1 ± 94.8 a	286.5 ± 32.0 abc
	VUltra	4.9 ± 0.3 a	5.5 ± 0.2 a	1.1 ± 0.0 ab	581.0 ± 17.8 b	817.5 ± 44.0 bc	260.2 ± 17.3 cd
	Fermaid E	4.8 ± 0.1 a	5.2 ± 0.1 b	1.0 ± 0.1 bc	570.6 ± 23.7 b	773.8 ± 70.4 c	242.8 ± 22.2 de
	OptiWhite	3.7 ± 0.1 b	3.3 ± 0.3 d	0.6 ± 0.1 de	342.3 ± 8.6 ef	422.2 ± 20.3 f	142.5 ± 12.3 fg
Sauvignon	DAP	3.5 ± 0.4 c	2.8 ± 0.5 e	0.4 ± 0.1 efg	208.2 ± 39.9 hi	335.3 ± 89.3 g	116.3 ± 37.5 gh
	VCombi	4.1 ± 0.3 b	3.7 ± 0.3 c	0.6 ± 0.1 de	295.9 ± 5.8 fg	536.3 ± 52.4 e	138.9 ± 31.9 g
	VUltra	3.8 ± 0.2 b	3.2 ± 0.0 de	0.4 ± 0.0 efg	257.4 ± 7.4 gh	427.8 ± 18.3 f	117.1 ± 7.7 gh
	Fermaid E	3.6 ± 0.0 b	3.1 ± 0.0 de	0.4 ± 0.0 efg	242.0 ± 13.7 h	414.8 ± 17.2 f	118.6 ± 12.7 g
	OptiWhite	3.1 ± 0.0 d	2.4 ± 0.0 f	0.3 ± 0.0 g	113.4 ± 11.1 j	205.7 ± 20.7 h	82.4 ± 10.4 h
X5	DAP	4.9 ± 0.1 a	5.4 ± 0.2 a	1.0 ± 0.1 bc	436.2 ± 7.1 d	873.3 ± 5.7 ab	306.0 ± 5.7 ab
	VCombi	5.1 ± 0.1 a	5.8 ± 0.2 a	1.2 ± 0.0 a	487.8 ± 9.6 c	954.3 ± 21.2 a	317.5 ± 28.4 a
	VUltra	4.4 ± 0.1 b	4.7 ± 0.0 b	1.0 ± 0.1 bc	375.3 ± 3.6 e	748.5 ± 7.9 c	271.3 ± 4.9 bcd
	Fermaid E	4.0 ± 0.1 b	4.0 ± 0.2 c	0.7 ± 0.1 d	307.4 ± 21.4 fg	649.6 ± 52.1 d	221.0 ± 8.8 e
	OptiWhite	3.4 ± 0.0 d	2.9 ± 0.0 de	0.5 ± 0.1 ef	168.6 ± 5.5 i	289.6 ± 17.4 g	119.5 ± 7.3 g

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

4.3.3 Effect of five commercial *Saccharomyces* yeast strains and two different commercial nutrient sources at different concentrations on growth kinetics, fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

The fermentation trial was performed in yellow passion fruit (YPF) juice, which was prepared from frozen yellow passion fruit puree in 2009. The chemical composition and volatile compounds in yellow passion puree and juice used in the fermentation trial were examined and the investigated results are given in **Table 4-23**.

The fermentation trial was performed in prepared YPF juice. Five commercial yeast strains; EC1118, VL3, X5, Alchemy I and LittoLevure and two different commercial nutrient sources; DAP and Vitamon Combi (VCombi) at two different concentrations; 0.25 and 0.5 g L^{-1}, were used for the fermentations of this YPF trial. In the previous YPF trial, the basic understanding of commercial yeast strain and nutrient supplementation in YPF wine fermentation were examined. Due to the fact that the addition of DAP, that is widely used in fruit wine production, to the YPF juice resulted in higher production of keto acids, hydrogen sulphide and some higher alcohols in the final YPF wines. Hence, the addition of a nutrient mixture containing thiamine (VCombi) with varying concentrations was investigated in order to optimize those compounds above.

This trial was aimed to investigate the effect of two nutrient sources with and without thiamine on the fermentation kinetics and performances, some metabolic and volatile compounds in final YPF wines. Four *Saccharomyces* yeast strains, VL3, X5, Alchemy I and LittoLevure were chosen on the basis of the results obtained from the previous trial of grape wine and YPF wine fermentations because their fermentation performances are considered to be more susceptible to nitrogen deficiency and composition. However, they also seemed to develop desirable fermentative characteristics and important volatile compounds in the final grape and YPF wines under certain conditions. Strain EC1118 has a high popularity in wine industry and fruit wine production, additionally it tended to produce less undesirable components, thus it was used as a control strain in this YPF fermentation trial.

YPF puree: YPF puree contained high acid namely 40.50 $\pm$ 0.00 g L^{-1} citric acid, 5.23 $\pm$ 0.00 g L^{-1} malic acid and had low pH at 2.9 $\pm$ 0.1 (**Table 4-23**). The other physico-chemical composition and aromas were TSS 9.0 $\pm$ 0.1 ^{0}Brix, 53.5 $\pm$ 3.5 g L^{-1} inverted sugar (as glucose), 296.0 $\pm$ 0.7 mg L^{-1} α-amino nitrogen/NOPA (as isoleucine), 2491.0 mg L^{-1} total amino acid without proline (**Table A-3**), 3.9 $\pm$ 0.1 mg L^{-1} 3-methyl butanol, 938.3 $\pm$ 67.3 μg L^{-1} hexanol, 141.6 $\pm$ 5.9 μg L^{-1} butanoic acid ethyl ester, 48.1 $\pm$ 1.3 μg L^{-1} linalool and 49.1

± 0.5 μg L^{-1} α-terpineol. Concentrations of 2-methyl butanol, 2-phenyl ethanol, acetic acid ethyl ester, propionic acid ethyl ester, succinic acid diethyl ester and ethyl ester of medium-chain fatty acids (MCFAs) were detected in trace quantities only (**Table 4-23**).

Table 4-23 Physico-chemical composition and some aromas of YPF puree and prepared YPF juice used in this fermentation trial

Compositions	YPF puree	Prepared YPF juice
pH	2.9 ± 0.1	3.2 ± 0.1
Total soluble solid (^{o}Brix)	9.0 ± 0.1	20.9 ± 0.5
Inverted sugar (g L^{-1})	53.5 ± 3.5	170.5 ± 12.1
NOPA (as mg L^{-1} isoleucine)	296.0 ± 0.7	24.0 ± 0.0
Total amino nitrogen without proline (mg L^{-1})	2491.0	123.1
Free SO_2 (mg L^{-1})	n.m.	2.6 ± 0.2
Total SO_2 (mg L^{-1})	n.m.	6.8 ± 0.8
Citric acid (g L^{-1})	40.50 ± 0.00	3.10 ± 0.62
Malic acid (g L^{-1})	5.23 ± 0.00	0.45 ± 0.06
3-Methyl butanol (mg L^{-1})	3.9 ± 0.1	n.d.
2-Methyl butanol (mg L^{-1})	traces	n.d.
2-Phenyl ethanol (mg L^{-1})	traces	n.d.
Hexanol (μg L^{-1})	938.3 ± 67.3	163.9 ± 2.7
Acetic acid ethyl ester (mg L^{-1})	traces	Traces
Propionic acid ethyl ester (μg L^{-1})	traces	n.d.
Butanoic acid ethyl ester (μg L^{-1})	141.6 ± 5.9	Traces
Succinic acid diethyl ester (μg L^{-1})	traces	n.d.
hexanoic acid ethyl ester (μg L^{-1})	traces	n.d.
octanoic acid ethyl ester (μg L^{-1})	traces	Traces
decanoic acid ethyl ester (μg L^{-1})	traces	Traces
Linalool (μg L^{-1})	48.1 ± 1.3	n.d.
α-Terpineol (μg L^{-1})	49.1 ± 0.5	6.9 ± 0.3

Each value shows the mean ± standard deviation from two sample replicates.

NOPA denotes free α-amino acid nitrogen

n.m. denotes not measured

n.d. denotes not detected

Prepared YPF juice: In **Table 4-23** and **Table A-3**, the physico-chemical properties of prepared YPF juice were pH 3.2 ± 0.1, 3.10 ± 0.62 g L^{-1} citric acid, 0.45 ± 0.06 g L^{-1} malic acid, TSS 20.9 ± 0.5 ^{0}Brix, 170.5 ± 12.1 g L^{-1} inverted sugar, 24.0 ± 0.0 mg L^{-1} α-amino nitrogen/NOPA, 123.1 mg L^{-1} total amino acid without proline, 163.9 ± 2.7 μg L^{-1} hexanol and 6.9 ± 0.3 μg L^{-1} α-terpineol. Concentrations of acetic acid ethyl ester, butanoic acid ethyl ester, octanoic acid and decanoic acid ethyl ester were only present in trace quantities.

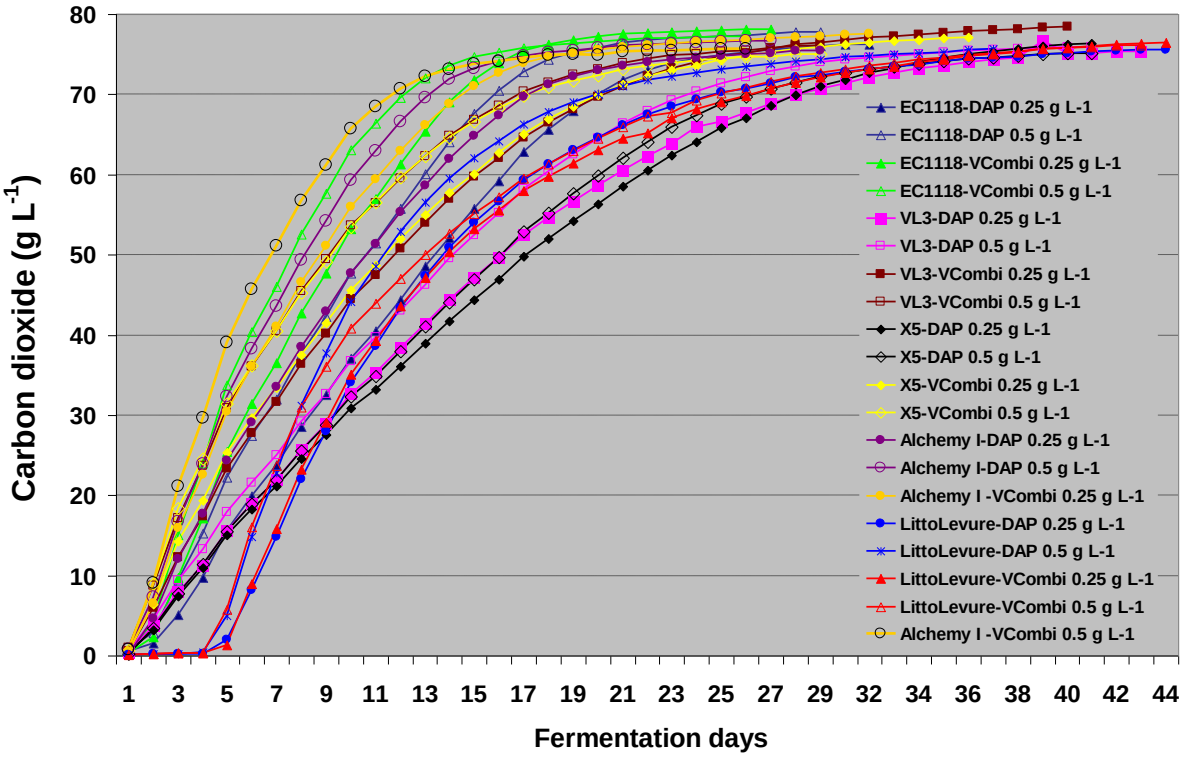


Figure 4-43 Growth kinetics of YPF juice fermentations with five different commercial yeast strains and two different nutrient sources at two different concentrations

The growth kinetics: As shown in **Figure 4-43**, the EC1118 and Alchemy I strain appeared to have similar fast fermentation kinetic and time, while the LittoLevure seemed to be the weakest strain, however the kinetics varied depending on nutrient supplementation. For strain LittoLevure, addition of any nutrient source and concentration resulted in the similar initial long lag phase for 4 days, but final fermentation times differed depending on the nutrient source and level. Strain LittoLevure had the longest fermentation duration in the presence of DAP or VCombi at low level (44 and 44 days, respectively). The addition of VCombi at both levels (0.25 and 0.5 g L^{-1}) enhanced strain EC1118 to have the fastest growth rates and fermentation times (26-27 days), on the contrary the similar nutrient addition resulted in slow and sluggish fermentations of strain LittoLevure. The fermentation kinetics and durations of strains Alchemy I, VL3 and X5 were accelerated in the high VCombi

treatment (26, 29 and 29 days, respectively). A high level of DAP addition also enhanced the fast kinetics of strains Alchemy I and EC1118 (27 and 29 days, respectively), whereas both levels of DAP addition resulted in slow and sluggish fermentations of strains VL3 and X5 (41 and 43 days, respectively). However, all YPF fermentations completely finished. In all cases, fermentation kinetics seemed to increase and the duration of fermentation decreased in response to increased nutrient concentrations, particularly in the VCombi treatment.

Chemical composition: In **Table 4-24,** the concentration of residual sugar was most present in YPF wines produced by strains X5 and Alchemy I with DAP addition (1.0 $\pm$ 0.2 and 1.1 $\pm$ 0.3 g L^{-1}, respectively) and by strain LittoLevure with VCombi treatment (1.1 $\pm$ 0.1 g L^{-1}). In addition, the five yeast strains practically almost consumed the initial sugar provided (**Table 4-23**) and resulted in low residual sugar below 2 g L^{-1}. Some differences existed between the YPF wine treatments in term of glycerol production. A low level of DAP promoted the VL3 and X5 strains to produce the highest amounts of glycerol (4.8 $\pm$ 0.5 and 4.9 $\pm$ 0.2 g L^{-1}, respectively), while strain Alchemy I produced the greatest level in high VCombi treatment (4.7 $\pm$ 0.4 g L^{-1}).

Organic acids: **Table 4-25** shows that all YPF wine treatments had similar amounts of acetic acid ranging from 0.20 $\pm$ 0.00 to 0.33 $\pm$ 0.05 g L^{-1}. The concentration of citric acid was most evident in the YPF wine produced by Alchemy I in the DAP sample at both levels (2.43 $\pm$ 0.10 and 2.47 $\pm$ 0.10 g L^{-1}, respectively) and at high level of VCombi (2.43 $\pm$ 0.10 g L^{-1}). Strain EC1118 also produced YPF wine having a high amount of this acid in the VCombi treatment at both levels (2.43 $\pm$ 0.10 and 2.43 $\pm$ 0.10 g L^{-1}, respectively). There was a slight decrease of citric acid at approximately 20.3-36.4 % in YPF wines. The highest amounts of malic acid were produced by strain LittoLevure in the high VCombi and low DAP treatment (0.78 $\pm$ 0.04 and 0.73 $\pm$ 0.01 g L^{-1}, respectively).

SO_2-binding compounds: **Figure 4-44** shows that the five yeast strains produced similar concentration of acetaldehyde ranged from 15.6 $\pm$ 0.6 to 24.2 $\pm$ 0.2 mg L^{-1}. Strain VL3 produced the greatest amount of α-ketoglutarate in the high DAP sample, followed by the low DAP addition (272.8 $\pm$ 5.6 and 251.7 $\pm$ 6.2 mg L^{-1}, respectively). Strain X5 also produced a high amount of this compound in both levels of DAP (246.2 $\pm$ 3.5 and 245.5 $\pm$ 5.1 mg L^{-1}, respectively) and the highest amount of pyruvate in the low level of DAP addition (156.6 $\pm$ 3.7 mg L^{-1}). It is likely that the addition of DAP resulted in excess keto acid production by strains VL3 and X5, however concentrations of these keto acids significantly diminished with the addition of VCombi to the YPF juice.

Table 4-24 Chemical composition of finished YPF wines obtained from YPF juices fermented with five different yeast strains with two nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	Residual sugar ($g\ L^{-1}$)	Glycerol ($g\ L^{-1}$)
EC1118	DAP	0.25	0.3 ± 0.1 f	4.0 ± 0.3 cde
		0.50	0.4 ± 0.2 ef	4.1 ± 0.4 bcde
	VCombi	0.25	0.4 ± 0.0 def	3.9 ± 0.5 de
		0.50	0.5 ± 0.0 cdef	4.0 ± 0.3 cde
VL3	DAP	0.25	0.8 ± 0.2 bc	4.8 ± 0.5 ab
		0.50	0.3 ± 0.1 f	4.0 ± 0.6 cde
	VCombi	0.25	0.4 ± 0.1 def	4.2 ± 0.5 bcde
		0.50	0.6 ± 0.1 cdef	4.5 ± 0.4 abcd
X5	DAP	0.25	1.0 ± 0.2 a	4.9 ± 0.2 a
		0.50	0.5 ± 0.2 cdef	3.8 ± 0.3 e
	VCombi	0.25	0.7 ± 0.2 bcde	4.1 ± 0.2 bcde
		0.50	0.6 ± 0.1 cdef	4.3 ± 0.1 abcde
Alchemy I	DAP	0.25	1.1 ± 0.3 a	4.5 ± 0.4 abcd
		0.50	0.7 ± 0.1 bcde	4.5 ± 0.2 abcd
	VCombi	0.25	0.6 ± 0.1 cdef	4.2 ± 0.5 bcde
		0.50	0.6 ± 0.2 cdef	4.8 ± 0.4 ab
LittoLevure	DAP	0.25	0.8 ± 0.1 bc	4.0 ± 0.3 cde
		0.50	0.5 ± 0.1 cdef	4.7 ± 0.4 abc
	VCombi	0.25	1.1 ± 0.1 a	4.4 ± 0.5 abcde
		0.50	0.5 ± 0.2 cdef	4.4 ± 0.5 abcde

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Table 4-25 Concentration of organic acids found in finished YPF wines produced by five different yeast strains with two nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	Citric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Acetic acid ($g\ L^{-1}$)
EC1118	DAP	0.25	2.13 ± 0.10 g	0.51 ± 0.01 h	0.27 ± 0.05 a
		0.50	2.37 ± 0.20 abc	0.48 ± 0.01 hj	0.30 ± 0.00 a
	VCombi	0.25	2.43 ± 0.10 ab	0.43 ± 0.02 ij	0.33 ± 0.05 a
		0.50	2.43 ± 0.10 ab	0.41 ± 0.01 j	0.30 ± 0.10 a
VL3	DAP	0.25	1.97 ± 0.10 h	0.68 ± 0.01 bcd	0.20 ± 0.00 a
		0.50	2.13 ± 0.10 g	0.72 ± 0.01 bc	0.30 ± 0.00 a
	VCombi	0.25	2.20 ± 0.00 efg	0.64 ± 0.02 de	0.23 ± 0.05 a
		0.50	2.27 ± 0.10 def	0.58 ± 0.01 fg	0.30 ± 0.00 a
X5	DAP	0.25	2.20 ± 0.00 efg	0.61 ± 0.01 ef	0.30 ± 0.00 a
		0.50	2.20 ± 0.00 efg	0.64 ± 0.02 de	0.30 ± 0.00 a
	VCombi	0.25	2.30 ± 0.00 cde	0.69 ± 0.01 ef	0.23 ± 0.03 a
		0.50	2.33 ± 0.10 bcd	0.54 ± 0.01 gh	0.30 ± 0.00 a
Alchemy I	DAP	0.25	2.43 ± 0.10 ab	0.50 ± 0.02 h	0.30 ± 0.00 a
		0.50	2.47 ± 0.10 a	0.48 ± 0.02 hi	0.30 ± 0.00 a
	VCombi	0.25	2.20 ± 0.00 fg	0.61 ± 0.01 ef	0.30 ± 0.00 a
		0.50	2.43 ± 0.10 ab	0.53 ± 0.02 gh	0.20 ± 0.00 a
LittoLevure	DAP	0.25	2.00 ± 0.00 h	0.73 ± 0.01 ab	0.20 ± 0.00 a
		0.50	2.23 ± 0.10 efg	0.67 ± 0.02 cd	0.20 ± 0.00 a
	VCombi	0.25	2.03 ± 0.10 h	0.71 ± 0.01 bc	0.23 ± 0.05 a
		0.50	2.03 ± 0.10 h	0.78 ± 0.04 a	0.27 ± 0.04 a

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

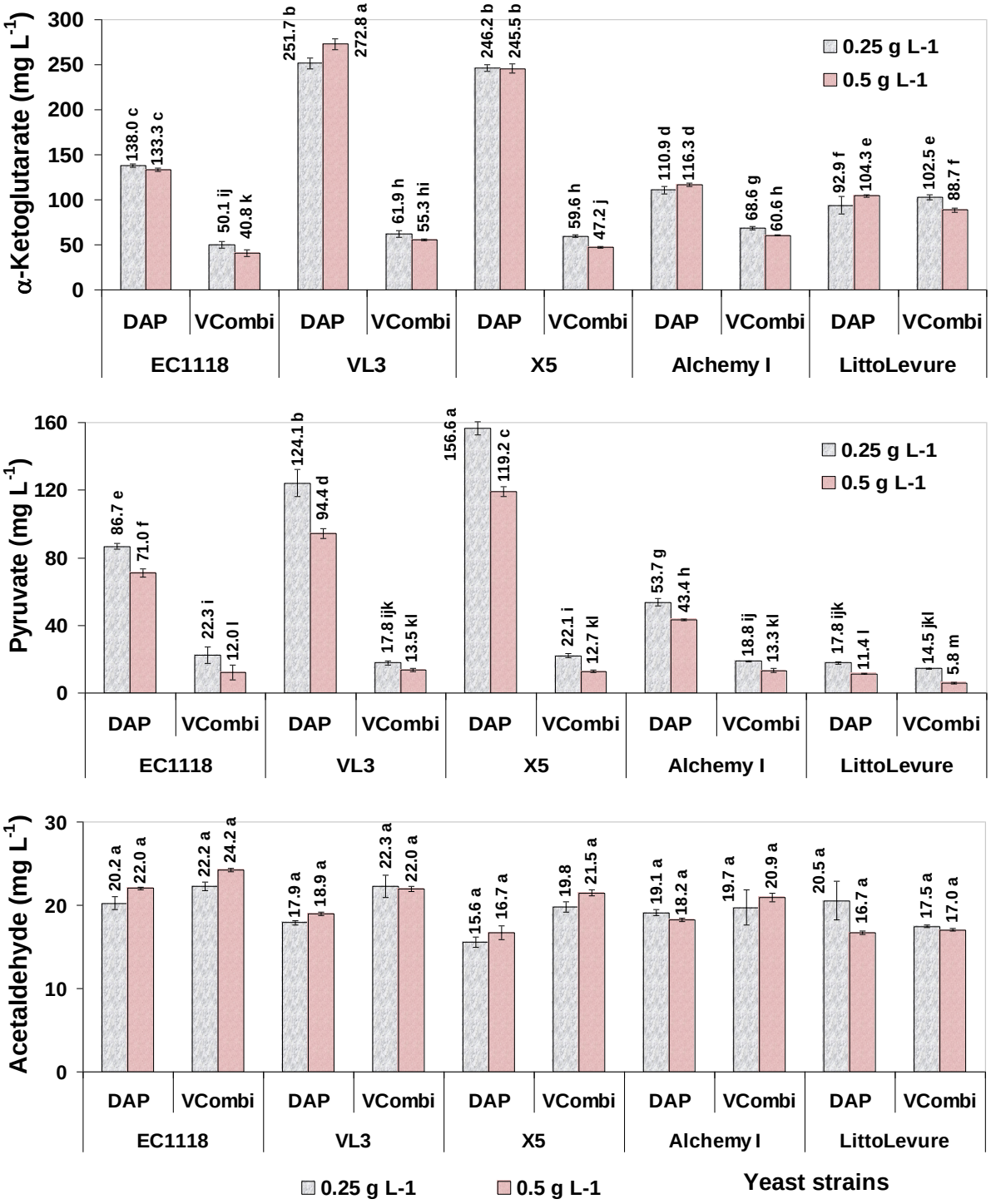


Figure 4-44 Concentration of SO_2-binding compounds found in finished YPF wines produced by five different yeast strains with two nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound, whereas those followed by same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

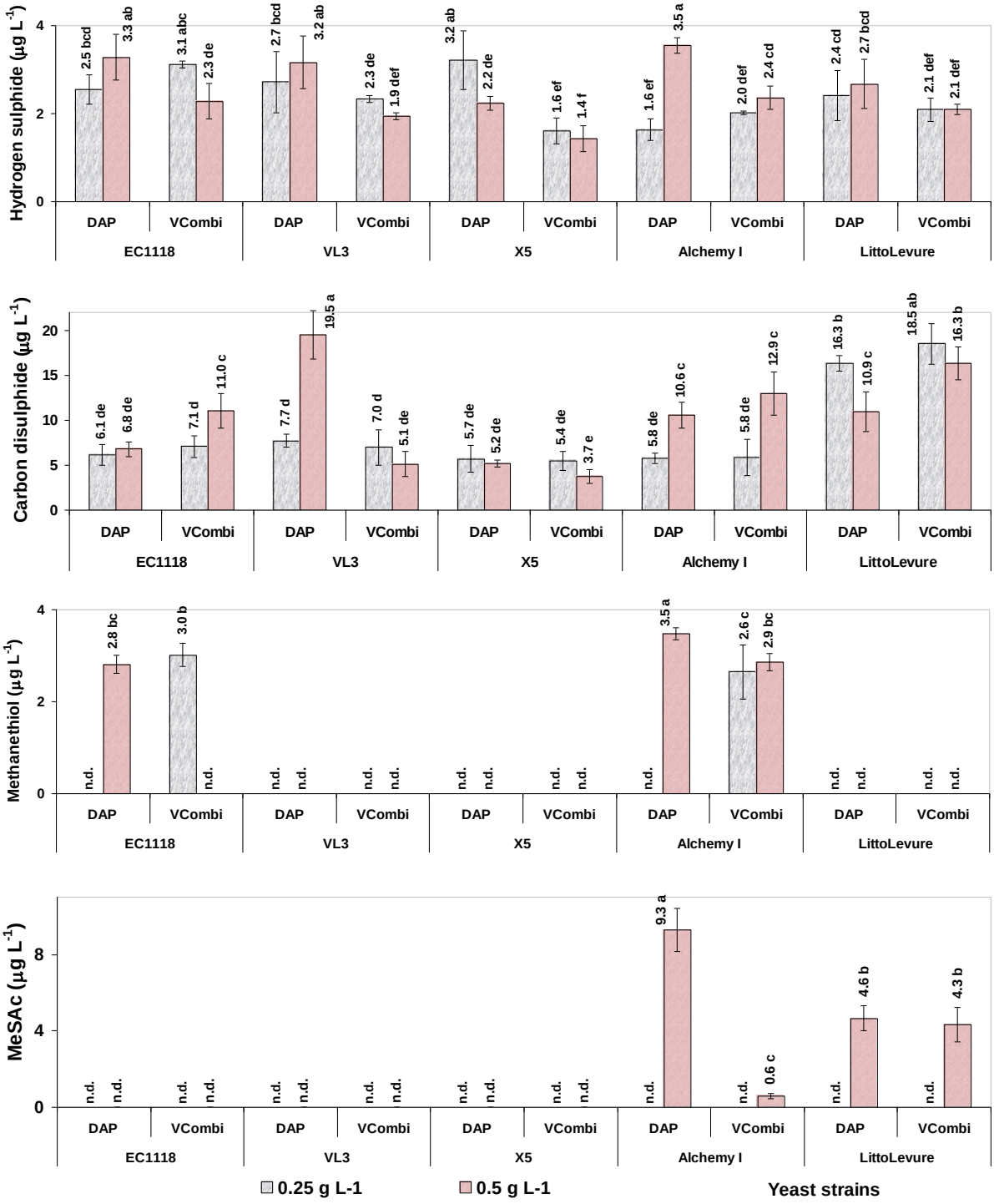


Figure 4-45 Concentration of hydrogen sulphide, carbon disulphide, methanethiol and MeSAc found in finished YPF wines produced by five different yeast strains with two nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

n.d. denotes not detected

Sulphur containing compounds: As shown in **Figure 4-45**, A high level of DAP addition stimulated the Alchemy I, EC1118 and VL3 strains to produce the highest amount of hydrogen sulphide (3.5 ± 0.2, 3.3 ± 0.5 and 3.2 ± 0.6 μg L^{-1}, respectively), on the contrary strain X5 produced the greatest amount in the low DAP sample (3.2 ± 0.7 μg L^{-1}). The highest concentrations of carbon disulphide were produced by strain VL3 in the high DAP treatment (19.5 ± 2.7 μg L^{-1}) and by strain LittoLevure in the low VCombi addition (18.5 ± 2.3 μg L^{-1}). For methanethiol, a high level of DAP promoted the highest formation by strain Alchemy I (3.5 ± 0.1 μg L^{-1}) followed by the EC1118 in the low VCombi treatment (3.0 ± 0.3 μg L^{-1}). Methanethiol was not detected in the YPF wines fermented with VL3, X5 and LittoLevure strains. The concentration of MeSAc was most evident in the YPF wines of the Alchemy I strain in the high DAP sample (9.3 ± 1.1 μg L^{-1}) followed by the LittoLevure YPF wine with the addition of DAP and VCombi at a high level (4.6 ± 0.6 and 4.3 ± 0.9 μg L^{-1}, respectively). Nevertheless, this sulphur containing ester was not detected in YPF wines from the EC1118, VL3 and X5 strains. Other sulphur compounds, ethanethiol, dimethyl sulphide, dimethyl disulphide, thioacetic acid S-ethylester, diethyl disulphide and dimethyl trisulphide were not detected in YPF wines.

Higher alcohols: The influence of five commercial yeast strains on the productions of these alcohols was dependent upon the nitrogen source and concentration, except for hexanol (**Figure 4-46**). The LittoLevure strain developed the highest amounts of 2-phenyl ethanol in both levels of VCombi addition and the high DAP sample (57.7 ± 3.6, 54.9 ± 4.5 and 54.4 ± 1.1 mg L^{-1}, respectively), while 3-methyl butanol and 2-methyl butanol were most evident in the high DAP treatment (224.5 ± 52.2 and 46.3 ± 12.5 mg L^{-1}, respectively). Addition of DAP at either a low or a high level promoted the highest formation of 2-methyl propanol by strain VL3 (108.4 ± 6.4 and 113.2 ± 4.2 mg L^{-1}, respectively) followed by strain EC1118 in the high DAP sample (74.0 ± 6.3 mg L^{-1}). Whereas, the addition of VCombi at both levels significantly reduced the production of this alcohol for all yeast strains. For hexanol, all YPF wines had similar amounts ranging from 166.0 ± 4.7 to 184.6 ± 7.6 μg L^{-1}.

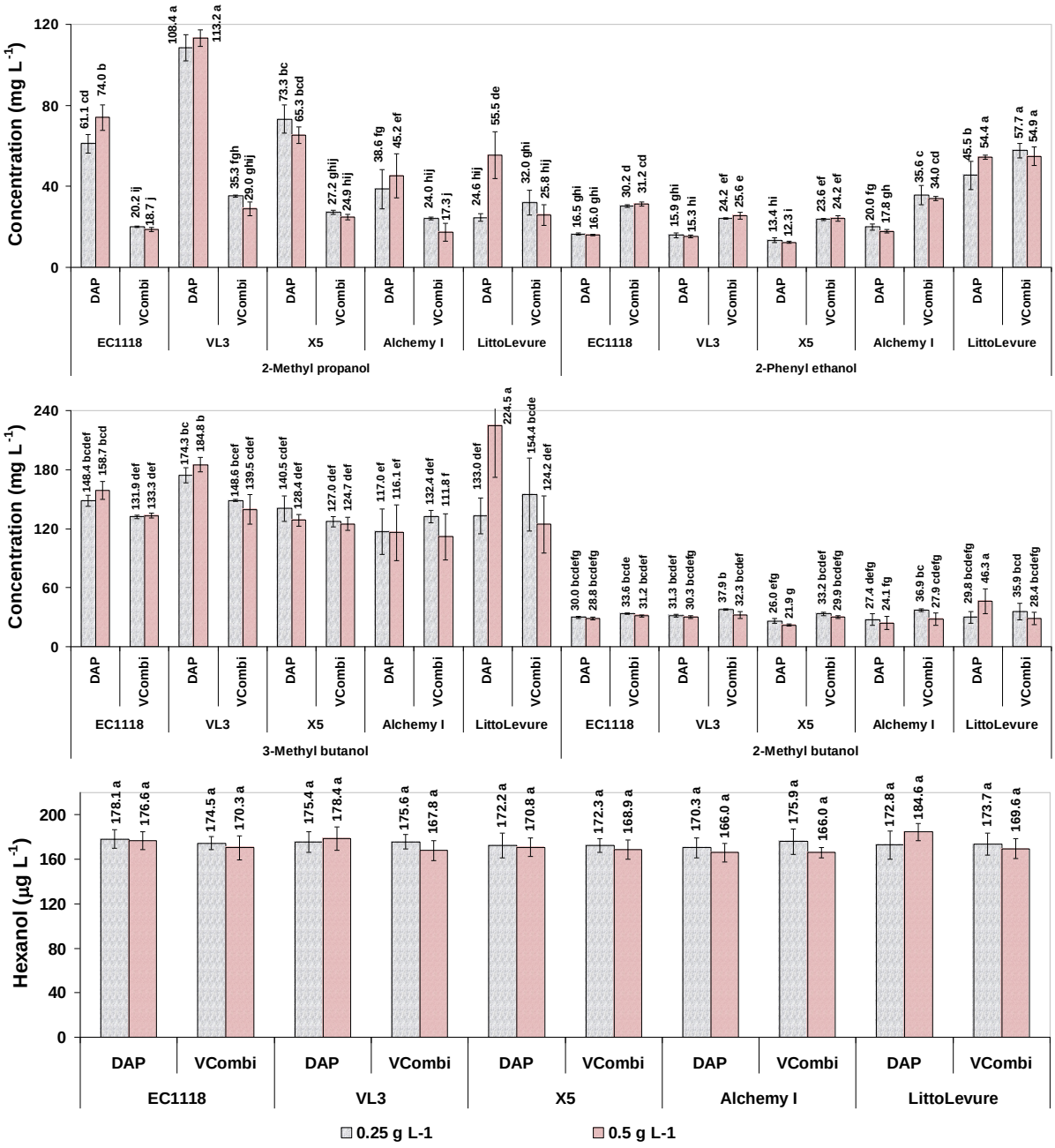


Figure 4-46 Concentration of higher alcohols found in finished YPF wines developed by five different yeast strains with two nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound, whereas those followed by same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

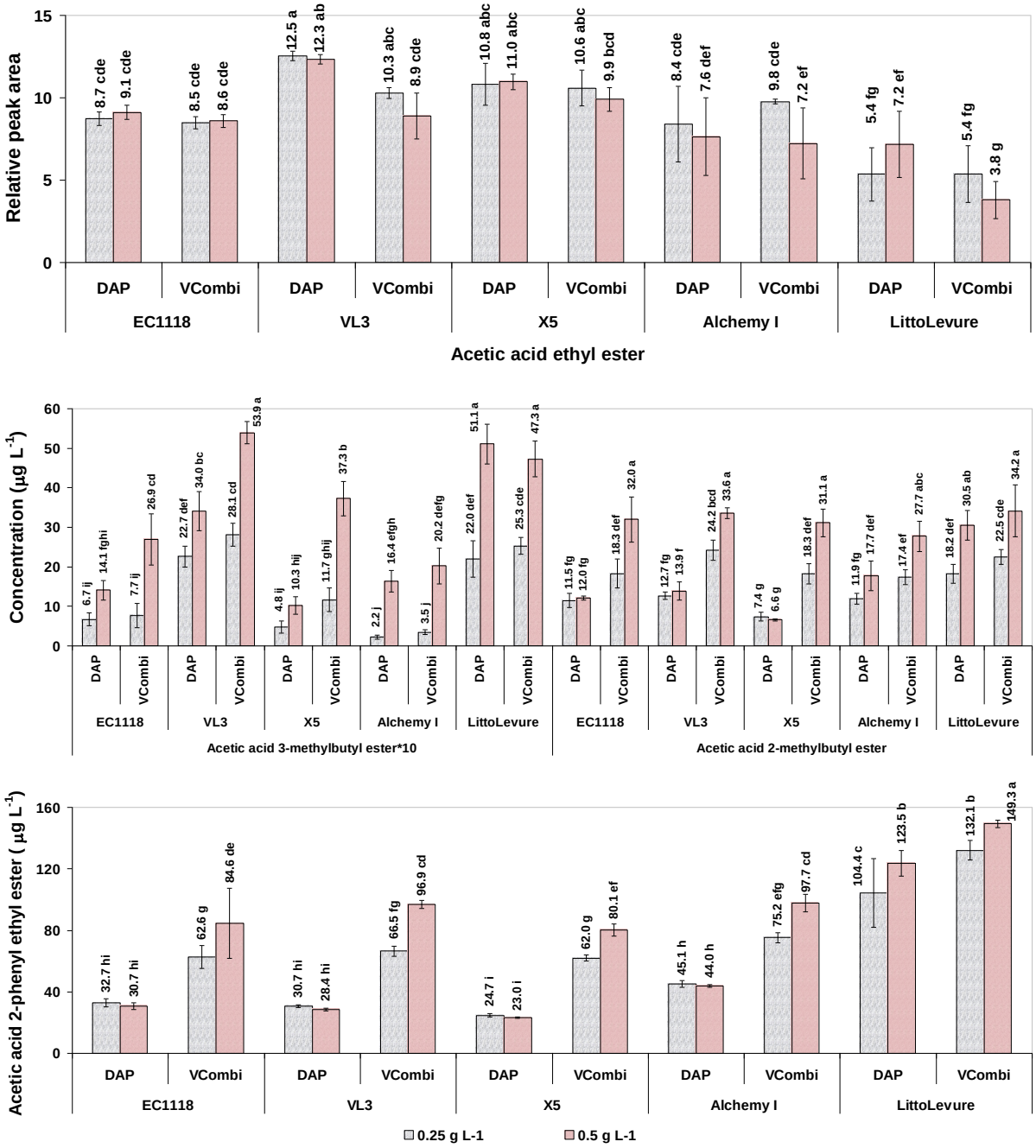


Figure 4-47 Concentration of acetic acid esters found in finished YPF wines developed by five different yeast strains with two nutrient sources at two different concentrations (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

Relative peak area = (peak area of sample)/(Peak area of internal standard)

Acetic acid esters: The five commercial yeast strains produced different amounts of acetic acid esters in response to nitrogen supplementation (**Figure 4-47**), whereas acetic acid hexyl ester was not detected in all YPF wines. Concentrations of acetic acid ethyl ester in YPF wine were below the detectable limit of the calibration standard value, therefore they were present as relative peak area. Addition of DAP at both levels promoted the highest production of acetic acid ethyl ester by strain VL3 (12.5 $\pm$ 0.3, and 12.3 $\pm$ 0.3 relative peak area, respectively) and by strain X5 (10.8 $\pm$ 1.3 and 11.0 $\pm$ 0.5 relative peak area, respectively). Strain LittoLevure developed the greatest concentrations of acetic acid 3-methylbutyl ester in the DAP and VCombi treatments at high level (510.8 $\pm$ 49.9 and 472.5 $\pm$ 45.1 $\mu g\ L^{-1}$, respectively) and acetic acid 2-methylbutyl ester in the high VCombi sample (34.2 $\pm$ 6.6 $\mu g\ L^{-1}$). Interestingly, it also produced the highest amount of acetic acid 2-phenyl ethyl ester in the high VCombi sample (149.3 $\pm$ 2.5 $\mu g\ L^{-1}$) followed by the low VCombi and high DAP treatments (132.1 $\pm$ 6.2 and 123.5 $\pm$ 8.4 $\mu g\ L^{-1}$). Concentration of acetic acid 3-methylbutyl ester was also most evident in the YPF wine produced by strain VL3 in the presence of high VCombi (539.1 $\pm$ 27.8 $\mu g\ L^{-1}$), while strains EC1118, VL3 and X5 also developed the greatest amount of acetic acid 2-methylbutyl ester in the same nutrient treatment (32.0 $\pm$ 5.7, 33.6 $\pm$ 1.3 and 31.1 $\pm$ 3.5 $\mu g\ L^{-1}$).

Ethyl and diethyl esters: The data in **Table 4-26** show that the interaction of yeast strain, nutrient source and concentration significantly affected the production of ethyl esters. The SAdiEtE was present in all YPF wines in trace quantities only. Strain X5 appeared to be a high producer of PrEtE with less response to nutrient addition (74.7 $\pm$ 5.3 to 97.8 $\pm$ 8.8 $\mu g\ L^{-1}$), whereas BuEtE was most evident in the high VCombi sample (157.4 $\pm$ 8.2 $\mu g\ L^{-1}$) and LAEtE in the high DAP sample (21.4 $\pm$ 1.0 $\mu g\ L^{-1}$). The highest concentration of PrEtE was also formed by the Alchemy I in the low VCombi treatment (93.3 $\pm$ 6.1 $\mu g\ L^{-1}$) and by strain VL3 in the high DAP addition (89.4 $\pm$ 5.3 $\mu g\ L^{-1}$). The VL3 strain also produced the greatest amount of LAEtE in the high DAP sample (21.4 $\pm$ 0.6 $\mu g\ L^{-1}$) and iBuEtE in the DAP treatment at both levels (58.0 $\pm$ 3.8 and 59.2 $\pm$ 5.9 $\mu g\ L^{-1}$, respectively). The concentration of BuEtE was also most evident in the YPF wine produced by strain EC1118 in the high VCombi sample (173.3 $\pm$ 42.2 $\mu g\ L^{-1}$). In most case, VCombi at both levels significantly decreased the concentrations of iBuEtE and LAEtE for most of the yeast strains.

Table 4-26 Concentration of ethyl and diethyl esters found in finished YPF wines produced by five different yeast strains with two nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations (g L^{-1})	PrEtE (μg L^{-1})	BuEtE (μg L^{-1})	iBuEtE (μg L^{-1})	LAEtE (mg L^{-1})	SAdiEtE (μg L^{-1})
EC1118	DAP	0.25	63.3 ± 2.1 cd	128.0 ± 10.7 bcd	14.6 ± 2.8 def	11.8 ± 0.6 d	trace
		0.50	70.1 ± 4.5 bc	146.1 ± 9.7 abc	18.7 ± 1.8 d	14.3 ± 0.7 c	trace
	VCombi	0.25	68.6 ± 11.2 bcd	128.5 ± 22.8 bcd	6.0 ± 1.8 h	7.9 ± 0.2 f	trace
		0.50	57.1 ± 7.6 cd	173.3 ± 42.2 a	5.5 ± 1.5 h	8.3 ± 0.2 f	trace
VL3	DAP	0.25	71.9 ± 2.3 bc	69.3 ± 2.9 gh	58.0 ± 3.8 a	16.7 ± 0.9 b	trace
		0.50	89.4 ± 5.3 ab	94.9 ± 4.7 efg	59.2 ± 5.9 a	21.4 ± 0.6 a	trace
	VCombi	0.25	78.5 ± 3.9 abc	108.8 ± 5.6 def	17.7 ± 1.1 d	8.0 ± 0.1 f	trace
		0.50	61.5 ± 4.4 cd	142.9 ± 5.7 bc	14.0 ± 1.7 def	8.4 ± 0.3 f	trace
X5	DAP	0.25	79.3 ± 8.1 abc	73.4 ± 5.1 gh	40.7 ± 2.0 b	17.3 ± 1.3 b	trace
		0.50	92.9 ± 1.7 ab	90.6 ± 2.7 efg	33.2 ± 0.8 c	21.4 ± 1.0 a	trace
	VCombi	0.25	97.8 ± 8.8 a	120.6 ± 12.9 cde	15.8 ± 0.4 de	7.9 ± 0.1 f	trace
		0.50	74.7 ± 5.3 abc	157.4 ± 8.2 ab	12.4 ± 0.8 efg	8.4 ± 0.2 f	trace
Alchemy I	DAP	0.25	77.0 ± 22.6 abc	66.9 ± 10.8 gh	15.3 ± 3.0 de	9.8 ± 0.2 e	trace
		0.50	57.4 ± 20.7 cd	112.4 ± 20.0 def	15.9 ± 2.6 de	11.1 ± 0.6 de	trace
	VCombi	0.25	93.3 ± 6.1 ab	82.1 ± 8.9 fgh	9.9 ± 0.8 fgh	8.4 ± 0.3 f	trace
		0.50	60.9 ± 19.8 cd	115.4 ± 10.5 cde	6.4 ± 1.5 h	8.3 ± 0.1 f	trace
LittoLevure	DAP	0.25	43.3 ± 17.4 de	54.2 ± 8.4 h	11.2 ± 3.0 efg	8.8 ± 0.4 f	trace
		0.50	55.6 ± 16.4 cd	97.2 ± 13.2 efg	16.2 ± 2.7 de	10.6 ± 0.5 de	trace
	VCombi	0.25	43.1 ± 12.3 de	55.8 ± 13.8 h	14.8 ± 4.0 de	8.8 ± 0.4 f	trace
		0.50	25.2 ± 2.7 e	76.0 ± 18.7 gh	8.9 ± 2.5 gh	8.9 ± 0.5 f	trace

Each value shows the mean ± standard deviation from three fermentation replicates. Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Medium-chain fatty acids and related ethyl esters: As given in **Table 4-27**, the results show that the production of MCFAs, except for decanoic acid, and MCFA ethyl esters significantly differed depending on yeast strains and nutrient supplementation. Strain EC1118 developed the highest amounts of hexanoic and octanoic acids in the high VCombi sample (4.3 ± 0.5 and 3.7 ± 0.8 mg L^{-1}, respectively). It also produced the highest amounts of HAEtE, OAEtE and DAEtE in both levels of VCombi treatments (369.4 ± 87.4 and 412.7 ± 85.9, 384.4 ± 45.8 and 480.5 ± 80.0, and 120.9 ± 29.3 and 139.4 ± 39.9 μg L^{-1}, respectively). The greatest concentrations of these compounds were also present in the YPF wines produced by strain X5 in a high VCombi treatment (4.1 ± 0.2 hexanoic acid, 3.5 ± 0.4 mg L^{-1} octanoic acid, 344.8 ± 25.5 μg L^{-1} HAEtE, 496.5 ± 82.0 μg L^{-1} OAEtE and 139.0 ± 7.3 μg L^{-1} DAEtE). The high VCombi addition also promoted the highest formation of all ethyl esters of MCFAs by the strain LittoLevure (369.4 ± 87.4, 384.4 ± 45.8 and 120.9 ± 29.3 μg L^{-1} HAEtE, OAEtE and DAEtE, respectively). Whereas, concentrations of decanoic acid in YPF wines were similar ranging from 0.10 ± 0.0 to 0.23 ± 0.1 mg L^{-1}.

Monoterpenes: **Figure 4-48** shows that under this trial condition, three yeast strains released similar concentrations of linalool and α-terpineol without any response to the nutrient addition. The YPF wines had similar concentrations of linalool ranging from 3.9 ± 0.1 to 6.1 ± 0.2 μg L^{-1} and α-terpineol from 8.7 ± 0.1 to 10.2 ± 0.1 μg L^{-1}. Other monoterpenes like *trans*-linalool oxide and *cis*-linalool oxide were not detected in all YPF wines.

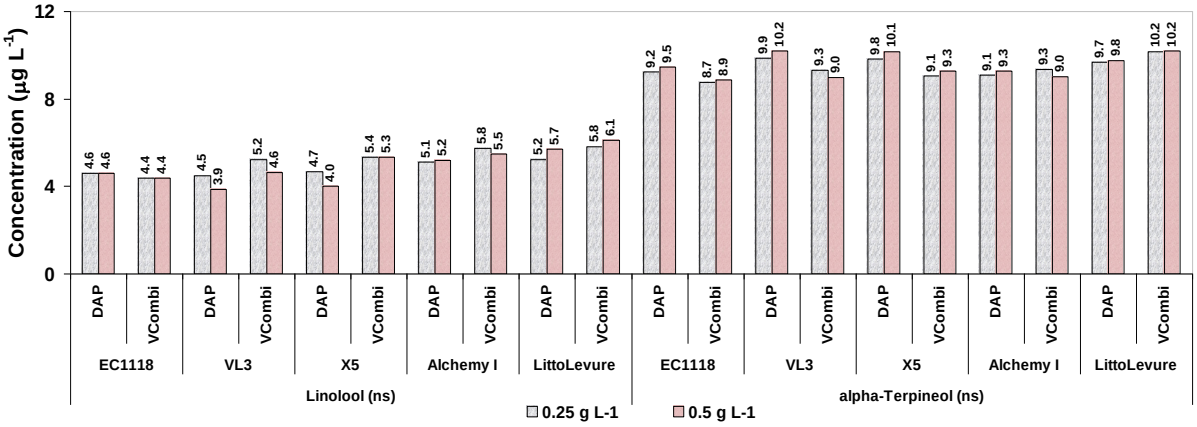


Figure 4-48 Concentration of linalool and α-terpineol found in finished YPF wines developed by five different yeast strains with two nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Compounds displaying **"(ns)"** show no significant difference (p>0.05) among treatment combinations of each compound according to the DMRT test.

Table 4-27 Concentration of fatty acids and their ethyl esters found in finished YPF wines produced by five different yeast strains with two nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	Hexanoic acid ($g\ L^{-1}$)	Octanoic acid ($g\ L^{-1}$)	Decanoic acid ($g\ L^{-1}$)	HAEtE ($\mu g\ L^{-1}$)	OAEtE ($\mu g\ L^{-1}$)	DAEtE ($\mu g\ L^{-1}$)
EC1118	DAP	0.25	3.3 ± 0.2 de	2.3 ± 0.3 def	0.10 ± 0.0 a	235.3 ± 38.1 bcd	267.4 ± 76.7 bcde	71.3 ± 18.2 def
		0.50	3.4 ± 0.2 cd	2.5 ± 0.3 cde	0.10 ± 0.0 a	213.1 ± 17.7 bcde	306.6 ± 69.5 bcd	78.0 ± 14.0 cde
	VCombi	0.25	3.8 ± 0.4 bc	3.1 ± 0.5 abc	0.17 ± 0.0 a	369.4 ± 87.4 a	384.4 ± 45.8 ab	120.9 ± 29.3 ab
		0.50	4.3 ± 0.5 a	3.7 ± 0.8 a	0.23 ± 0.1 a	412.7 ± 85.9 a	480.5 ± 80.0 a	139.4 ± 39.9 a
VL3	DAP	0.25	2.6 ± 0.2 e	1.5 ± 0.2 g	0.10 ± 0.0 a	47.5 ± 8.4 h	65.9 ± 25.9 h	26.3 ± 5.6 gh
		0.50	2.8 ± 0.2 de	1.6 ± 0.2 fg	0.10 ± 0.0 a	73.3 ± 13.2 h	135.9 ± 31.2 efgh	39.2 ± 3.9 gh
	VCombi	0.25	3.2 ± 0.2 de	2.1 ± 0.3 efg	0.10 ± 0.0 a	162.6 ± 34.9 def	248.8 ± 67.7 bcdef	71.9 ± 19.0 def
		0.50	3.7 ± 0.0 b	2.9 ± 0.2 bcd	0.13 ± 0.0 a	243.3 ± 23.5 bc	371.9 ± 61.9 abc	102.9 ± 13.0 bc
X5	DAP	0.25	2.8 ± 0.1 de	1.6 ± 0.1 efg	0.10 ± 0.0 a	83.3 ± 21.6 gh	66.0 ± 28.7 h	31.1 ± 7.4 gh
		0.50	2.8 ± 0.2 de	1.6 ± 0.2 fg	0.10 ± 0.0 a	84.1 ± 11.4 gh	113.5 ± 35.1 fgh	28.9 ± 6.2 gh
	VCombi	0.25	3.4 ± 0.1 cd	2.4 ± 0.3 cde	0.10 ± 0.0 a	265.1 ± 35.4 b	313.3 ± 61.3 bcd	90.0 ± 9.4 cd
		0.50	4.1 ± 0.2 ab	3.5 ± 0.4 ab	0.20 ± 0.0 a	344.8 ± 25.5 a	496.5 ± 82.0 a	139.0 ± 7.3 a
Alchemy I	DAP	0.25	2.8 ± 0.2 de	1.8 ± 0.3 efg	0.10 ± 0.0 a	113.6 ± 13.3 fgh	102.2 ± 30.0 gh	24.1 ± 1.9 h
		0.50	3.1 ± 0.2 de	2.3 ± 0.5 def	0.10 ± 0.0 a	166.7 ± 26.0 def	206.7 ± 35.9 defg	50.5 ± 4.1 efgh
	VCombi	0.25	2.9 ± 0.2 de	1.8 ± 0.3 efg	0.10 ± 0.0 a	150.3 ± 9.0 efg	163.7 ± 38.7 efgh	42.7 ± 5.9 fgh
		0.50	3.5 ± 0.2 bc	2.8 ± 0.6 cde	0.13 ± 0.0 a	252.0 ± 39.8 bc	305.3 ± 75.1 bcd	87.1 ± 10.6 cd
LittoLevure	DAP	0.25	3.2 ± 0.1 de	2.4 ± 0.2 def	0.13 ± 0.0 a	120.8 ± 11.4 fgh	200.7 ± 47.7 defgh	53.8 ± 10.6 efgh
		0.50	3.6 ± 0.0 b	2.8 ± 0.1 bcd	0.13 ± 0.0 a	235.3 ± 38.1 bcd	267.4 ± 76.7 bcde	71.3 ± 18.2 def
	VCombi	0.25	3.2 ± 0.2 de	2.3 ± 0.3 def	0.13 ± 0.0 a	213.1 ± 17.7 bcde	306.6 ± 69.5 bcd	78.0 ± 14.0 cde
		0.50	3.8 ± 0.2 b	3.2 ± 0.2 bcd	0.17 ± 0.1 a	369.4 ± 87.4 a	384.4 ± 45.8 ab	120.9 ± 29.3 ab

Each value shows the mean ± standard deviation. Values displaying the same letter (only 'a') within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

4.3.4 Effect of three commercial *Saccharomyces* yeast strains and four different nutrient sources at two different concentrations on growth kinetics, fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

The fermentation trial was performed in yellow passion fruit (YPF) juice, which was prepared from frozen YPF puree. The physico-chemical characteristics and volatile compounds in YPF puree and prepared YPF juice used in this fermentation trial are given in **Table 4-28**.

The fermentation trial was performed in prepared YPF juice. Three commercial yeast strains of EC1118, VL3 and X5; four commercial nutrient sources of Vitamon Combi (VCombi), Vitamon Ultra (VUltra), Fermaid E blanc (Fermaid E) and OptiWhite, and two concentrations at 0.2 and 0.4 g L^{-1}, were used in this YPF fermentation trial. This YPF fermentation trial was aimed to investigate the impact of yeast strains, nutrient source and concentration on the fermentation characteristics, non-volatile and volatile compounds.
Yeast strains and nutrient sources were obtained from the basis results of previous trials. The EC1118 strain has a high popularity in wine and fruit wine industry and is recommended for all types of wine. It also tended to produce less undesirable components under certain condition as shown in the previous trials. Hence, it was used as a control strain in this YPF trial, while the two *Saccharomyces* yeast strains, VL3 and X5 were chosen as they respond to the nutrient supplementation, which affects the fermentation characteristics. Under certain conditions, they also developed desirable fermentative characteristics and volatile compounds as shown in previous trials. In addition, they have shown to have differences in fermentation performances and organoleptic characteristics imparted to wines and also to produce low SO_2 and H_2S, but rather to develop varietal aroma (Eglinton & Henschke, 1996; Reynolds et al., 2001; Wang et al., 2003; Howell et al., 2004; Dubourdieu et al., 2006; Swiegers et al., 2007, 2009).

YPF Puree: The data in **Table 4-28** show that it had high acidity, which comprises mainly citric acid 42.10 $\pm$ 0.00 g L^{-1} and malic acid 5.25 $\pm$ 0.00 g L^{-1}, and low pH at 2.9 $\pm$ 0.1. The other chemical properties were TSS 9.0 $\pm$ 0.1 ^{0}Brix, 58.2 $\pm$ 1.5 g L^{-1} inverted sugar (as glucose), 296.0 $\pm$ 5.7 mg L^{-1} α-amino nitrogen/NOPA (as isoleucine) and 2491.0 mg L^{-1} total amino acid without proline (**Table A-3**). Some volatile compounds were 3.9 $\pm$ 0.0 mg L^{-1} 3-methyl butanol, 918.3 $\pm$ 1.9 μg L^{-1} hexanol, 154.2 $\pm$ 2.6 μg L^{-1} butanoic acid ethyl ester, 47.1 $\pm$ 0.6 μg L^{-1} linalool and 48.0 $\pm$ 0.2 μg L^{-1} α-terpineol. While, 2-methyl butanol, 2-phenyl ethanol, acetic acid ethyl ester, propionic acid ethyl ester, succinic acid diethyl ester and ethyl ester of medium-chain fatty acids were detected only in trace quantities.

Prepared YPF juice: The physico-chemical characteristics of prepared YPF juice were pH 3.2 ± 0.1, 2.80 ± 0.07 g L^{-1} citric acid, 0.51 ± 0.01 g L^{-1} malic acid, TSS 21.0 ± 0.6 ^{o}Brix, 170.0 ± 2.8 g L^{-1} inverted sugar (as glucose), 26.7 ± 1.2 mg L^{-1} α-amino nitrogen/NOPA and 164.4 mg L^{-1} total amino acid without proline (**Table 4-28**). The volatile components were 2.2 ± 0.3 mg L^{-1} free SO_2 and 6.9 ± 0.3 mg L^{-1} total SO_2, 177.7 ± 2.8 μg L^{-1}, hexanol and 7.4 ± 0.2 μg L^{-1} α-terpineol. Whereas, acetic acid ethyl ester, ethyl ester of butanoic acid, octanoic acid and decanoic acid were detected only in trace quantities, but the other aroma compounds that can be detected in YPF puree were not detected in the prepared YPF juice.

Table 4-28 Chemical compositions of YPF puree and prepared YPF juice used in this fermentation trial

Composition	YPF puree	Prepared YPF juice
pH	2.9 ± 0.1	3.2 ± 0.1
Total soluble solid (^{o}Brix)	9.0 ± 0.1	21.0 ± 0.6
Inverted sugar (g L^{-1})	58.2 ± 1.5	170.0 ± 2.8
NOPA (as mg L^{-1} isoleucine)	296.0 ± 5.7	26.7 ± 1.2
Total amino nitrogen without proline (mg L^{-1})	2491.0	164.4
Free SO_2 (mg L^{-1})	n.m.	2.2 ± 0.3
Total SO_2 (mg L^{-1})	n.m.	6.9 ± 0.5
Citric acid (g L^{-1})	42.10 ± 0.00	2.80 ± 0.07
Malic acid (g L^{-1})	5.25 ± 0.00	0.51 ± 0.01
3-Methyl butanol (mg L^{-1})	3.9 ± 0.0	n.d.
2-Methyl butanol (mg L^{-1})	traces	n.d.
2-Phenyl ethanol (mg L^{-1})	traces	n.d.
Hexanol (μg L^{-1})	918.3 ± 1.9	177.7 ± 2.8
Acetic acid ethyl ester (mg L^{-1})	traces	traces
Propionic acid ethyl ester (μg L^{-1})	traces	n.d.
Butanoic acid ethyl ester (μg L^{-1})	154.2 ± 2.6	traces
Succinic acid diethyl ester (μg L^{-1})	traces	n.d.
Octanoic acid ethyl ester (μg L^{-1})	traces	traces
Decanoic acid ethyl ester (μg L^{-1})	traces	traces
Linalool (μg L^{-1})	47.1 ± 0.6	n.d.
α-Terpineol (μg L^{-1})	48.0 ± 0.2	7.4 ± 0.2

Each value shows the mean ± standard deviation from two sample replicates.

NOPA denotes free α-amino acid nitrogen.

n.d. denotes not detected. n.m. denotes not measured

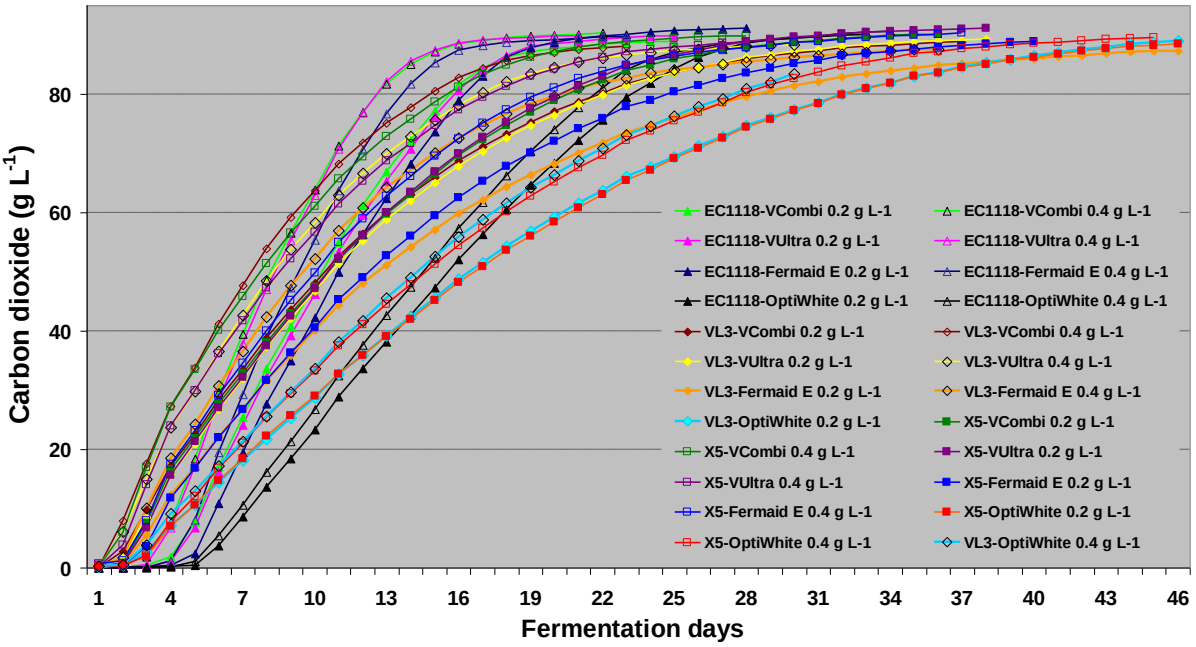


Figure 4-49 Growth kinetics of YPF juice fermentations with three different commercial yeast strains and four different nutrient mixtures at two different concentrations

The fermentation kinetics: In **Figure 4-49**, for strain EC1118, low level of all nutrients as well as high OptiWhite addition gave a similar long growth lag phase (4-5 days) and had slower growth kinetics than the other fermentations. However, it completely finished fermentation faster than the other yeast strains with similar nutrient conditions (25-33 days). On the other hand, the addition of high concentration of any kind of nutrient, except for the OptiWhite, enhanced the growth kinetics and reduced fermentation times from 25-34 days at low nutrient level to 21- 23 days. When any level of OptiWhite was added to the YPF juice, strain VL3 and X5 showed a similar long growth lag phase (3 days), followed by slow fermentation kinetics and duration, however they finished fermentations after 30-46 days. In addition, the fermentation with strain VL3 at high OptiWhite addition seemed to result in stuck fermentation with high residual sugar (**Table 4-29**). While, the supplementation of YPF juice with VCombi, VUltra and Fermaid E at either low or high level stimulated the VL3 and X5 strains to have similar faster growth kinetics, but they had afterwards different durations of fermentation in response to the nutrient addition. Strain VL3 had sluggish fermentations in a low level of VCombi, VUltra and Fermaid E (37-46 days), but at high level of these nutrients significantly decreased fermentation times to 23-36 days. The X5 strain followed similar pattern as strain VL3, but had shorter fermentation times in the same nutrient conditions.

Chemical composition: As shown in **Table 4-29**, the data show that the three yeast strains produced YPF wines having significantly different amounts of residual sugar in response to nutrient source and concentration. The fermentation with strain VL3 at high OptiWhite addition resulted in the highest level of residual sugar in YPF wine (10.08 $\pm$ 0.3 g L^{-1}), followed by strain VL3 and X5 at low OptiWhite treatment (2.82 $\pm$ 0.6 and 3.0 $\pm$ 0.3 g L^{-1}, respectively). While, the EC1118 strain seemed to produce YPF wines having similar low amounts of residual sugar in most nutrients (0.30 $\pm$ 0.2 to 0.50 $\pm$ 0.1 g L^{-1}), except in the low OptiWhite sample (0.75 $\pm$ 0.2 g L^{-1}). The YPF wines had similar concentrations of glycerol, which ranged from 2.8 $\pm$ 0.2 to 5.6 $\pm$ 0.4 g L^{-1}.

Organic acids: Yeast strain, nutrient source and concentration significantly affected the concentrations of malic and citric acid in YPF wines (**Table 4-30**). Concentrations of acetic acid had no statistical differences among the YPF wine treatments ranging from 0.10 $\pm$ 0.00 to 0.43 $\pm$ 0.07 g L^{-1}. The highest amounts of malic acid were found in the YPF wine of strain VL3 at both levels of Fermaid E (0.79 $\pm$ 0.02 and 0.76 $\pm$ 0.01 g L^{-1}, respectively) and strain X5 in the low Fermaid E treatment (0.77 $\pm$ 0.01 g L^{-1}). The two strains above can catalyze malic acid, particularly strain VL3 produced the highest amounts (32.9-35.4 %). Although strain EC1118 seemed to have no capability to produce this organic acid, it produced YPF wines having the highest concentration of citric acid in the high Fermaid E and VCombi samples (2.73 $\pm$ 0.05 and 2.70 $\pm$ 0.00 g L^{-1}, respectively), followed by the samples with low level of VCombi and VUltra additions (2.67 $\pm$ 0.05 and 2.67 $\pm$ 0.05 g L^{-1}).

SO_2-binding compounds: Three yeast strains, nutrient source and concentration statistically affected both keto acids but not acetaldehyde (**Figure 4-50**). There were similar concentrations of acetaldehyde among all YPF wines ranging from 24.5 $\pm$ 0.3 to 30.6 $\pm$ 0.9 mg L^{-1}. The addition of OptiWhite at low level stimulated the highest production of α-ketoglutarate by strain VL3 (163.2 $\pm$ 15.3 mg L^{-1}), followed by strain X5 (142.6 $\pm$ 5.3 mg L^{-1}), and the former strain in the high OptiWhite sample (137.0 $\pm$ 3.6 mg L^{-1}). The addition of YPF juice with OptiWhite at high level resulted in the highest formation of pyruvate by the VL3 strain (143.0 $\pm$ 2.9 mg L^{-1}), followed by the same nutrient at low level (126.3 $\pm$ 5.0 mg L^{-1}). Nevertheless, when the other nutrient sources were added, particularly at high level, the production of both keto acids significantly decreased.

Table 4-29 Chemical composition of final YPF wines obtained from YPF juices fermented with three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations (g L^{-1})	Residual sugar (g L^{-1})	Glycerol (g L^{-1})
EC1118	VCombi	0.2	0.32 ± 0.1 h	3.9 ± 0.7 a
		0.4	0.30 ± 0.2 h	3.5 ± 0.7 a
	VUltra	0.2	0.33 ± 0.0 gh	3.5 ± 0.8 a
		0.4	0.33 ± 0.0 gh	3.8 ± 0.6 a
	Fermaid E	0.2	0.50 ± 0.1 efgh	3.8 ± 0.5 a
		0.4	0.37 ± 0.1 fgh	3.9 ± 0.2 a
	OptiWhite	0.2	0.75 ± 0.2 cdef	3.8 ± 0.9 a
		0.4	0.45 ± 0.1 fgh	4.2 ± 0.6 a
VL3	VCombi	0.2	0.72 ± 0.1 cdefg	3.2 ± 0.1 a
		0.4	0.50 ± 0.1 efgh	3.9 ± 0.8 a
	VUltra	0.2	0.87 ± 0.3 cde	3.5 ± 0.6 a
		0.4	0.65 ± 0.2 defgh	3.0 ± 1.0 a
	Fermaid E	0.2	0.62 ± 0.0 defgh	4.2 ± 0.7 a
		0.4	0.45 ± 0.2 fgh	4.2 ± 0.2 a
	OptiWhite	0.2	2.82 ± 0.6 b	5.6 ± 0.4 a
		0.4	10.08 ± 0.3 a	5.0 ± 0.7 a
X5	VCombi	0.2	0.47 ± 0.2 fgh	3.5 ± 0.7 a
		0.4	0.47 ± 0.2 fgh	3.4 ± 0.5 a
	VUltra	0.2	0.42 ± 0.2 fgh	3.6 ± 0.5 a
		0.4	0.53 ± 0.1 defgh	3.3 ± 0.6 a
	Fermaid E	0.2	1.05 ± 0.1 c	2.8 ± 0.2 a
		0.4	0.53 ± 0.1 defgh	3.3 ± 0.5 a
	OptiWhite	0.2	3.00 ± 0.3 b	3.5 ± 0.2 a
		0.4	0.90 ± 0.3 cd	4.8 ± 0.5 a

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Table 4-30 Concentration of organic acids found in finished YPF wines produced by three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Acetic acid ($g\ L^{-1}$)	Citric acid ($g\ L^{-1}$)
EC1118	VCombi	0.2	0.48 ± 0.01 hij	0.27 ± 0.08 a	2.67 ± 0.05 abc
		0.4	0.45 ± 0.01 j	0.20 ± 0.00 a	2.70 ± 0.00 ab
	VUltra	0.2	0.47 ± 0.01 hij	0.37 ± 0.05 a	2.67 ± 0.05 abc
		0.4	0.46 ± 0.00 ij	0.30 ± 0.00 a	2.63 ± 0.05 bc
	Fermaid E	0.2	0.50 ± 0.00 h	0.37 ± 0.05 a	2.37 ± 0.07 ef
		0.4	0.45 ± 0.01 j	0.30 ± 0.00 a	2.73 ± 0.05 a
	OptiWhite	0.2	0.49 ± 0.01 hi	0.37 ± 0.06 a	2.40 ± 0.00 def
		0.4	0.50 ± 0.02 h	0.27 ± 0.05 a	2.47 ± 0.05 de
VL3	VCombi	0.2	0.72 ± 0.03 cd	0.27 ± 0.06 a	2.20 ± 0.00 hi
		0.4	0.63 ± 0.02 f	0.20 ± 0.00 a	2.60 ± 0.00 c
	VUltra	0.2	0.74 ± 0.03 bc	0.23 ± 0.05 a	2.20 ± 0.00 hi
		0.4	0.62 ±0.02 fg	0.10 ± 0.00 a	2.50 ± 0.00 d
	Fermaid E	0.2	0.79 ± 0.02 a	0.23 ± 0.05 a	2.43 ± 0.05 de
		0.4	0.76 ± 0.01 ab	0.20 ± 0.00 a	2.13 ± 0.07 i
	OptiWhite	0.2	0.62 ± 0.02 fg	0.43 ± 0.07 a	2.40 ± 0.00 def
		0.4	0.61 ± 0.04 fg	0.33 ± 0.06 a	2.30 ± 0.10 fg
X5	VCombi	0.2	0.70 ± 0.02 de	0.17 ± 0.05 a	2.47 ± 0.05 de
		0.4	0.59 ± 0.01 g	0.20 ± 0.00 a	2.43 ± 0.07 de
	VUltra	0.2	0.70 ± 0.00 de	0.27 ± 0.03 a	2.27 ± 0.05 gh
		0.4	0.62 ± 0.01 fg	0.20 ± 0.00 a	2.40 ± 0.00 def
	Fermaid E	0.2	0.77 ± 0.01 ab	0.23 ± 0.03 a	2.27 ± 0.06 gh
		0.4	0.68 ± 0.03 e	0.20 ± 0.02 a	2.47 ± 0.05 de
	OptiWhite	0.2	0.61 ±0.01 fg	0.30 ± 0.00 a	2.47 ± 0.03 de
		0.4	0.62 ± 0.01 fg	0.30 ± 0.00 a	2.40 ± 0.00 def

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying the same letter (only ‘a’) within the same column indicate no significant difference ($p>0.05$) among treatment combinations, whereas those displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

Sulphur containing compounds: **Figure 4-51** shows that under this trial conditions, ethanethiol, dimethyl sulphide, dimethyl disulphide, thioacetic acid of S-ethylester and S-methylester, diethyl disulphide and dimethyl trisulphide were not detected in YPF wines. Strain EC1118 produced H_2S in most nutrient treatments, but the highest amount was present in the low OptiWhite sample (2.9 $\pm$ 0.1 $\mu g\ L^{-1}$). The greatest amount of H_2S was also produced by strain X5 in high OptiWhite addition (3.1 $\pm$ 0.2 $\mu g\ L^{-1}$), whereas in the low Fermaid E and OptiWhite and high VUltra samples H_2S was not detected. Strain X5 produced the highest amount of carbon disulphide in the low Fermaid E sample (17.1 $\pm$ 2.2 $\mu g\ L^{-1}$), followed by the high OptiWhite and low VUltra treatments (11.4 $\pm$ 1.0 and 10.8 $\pm$ 0.1 $\mu g\ L^{-1}$, respectively). Methanethiol was detected only in the YPF wines produced by strain EC1118 with the addition of VUltra and Fermaid E at high level (2.6 $\pm$ 0.2 and 2.7 $\pm$ 0.2 $\mu g\ L^{-1}$, respectively).

Higher alcohols: The results in **Figure 4-52** show that there were no significant differences in the concentration of hexanol among all YPF wines ranging from 177.3 $\pm$ 2.8 to 193.9 $\pm$ 6.4 $\mu g\ L^{-1}$. The VL3 strain developed the greatest amount of 2-methyl propanol in the OptiWhite sample at low level (112.7 $\pm$ 8.3 mg L^{-1}) followed by the one at high level (82.7 $\pm$ 0.9 mg L^{-1}). While, the EC1118 strain significantly produced the lowest amounts of this alcohol (18.5 $\pm$ 0.9 to 22.4 $\pm$ 2.6 mg L^{-1}), except in the OptiWhite treatment, but it produced the highest amount of 2-phenyl ethanol in the Fermaid E treatments and the high VCombi sample (41.2 $\pm$ 0.4, 40.7 $\pm$ 2.0 and 41.6 $\pm$ 1.6 mg L^{-1}). Strain VL3 developed the highest amount of 3-methyl butanol in both levels of Fermaid E sample and the low OptiWhite treatment (202.3 $\pm$ 3.0, 205.3 $\pm$ 32.8 and 196.8 $\pm$ 12.4 mg L^{-1}, respectively), whereas 2-methyl butanol was most evident in both Fermaid E levels (52.3 $\pm$ 1.4 and 51.2 $\pm$ 8.1 mg L^{-1}, respectively).

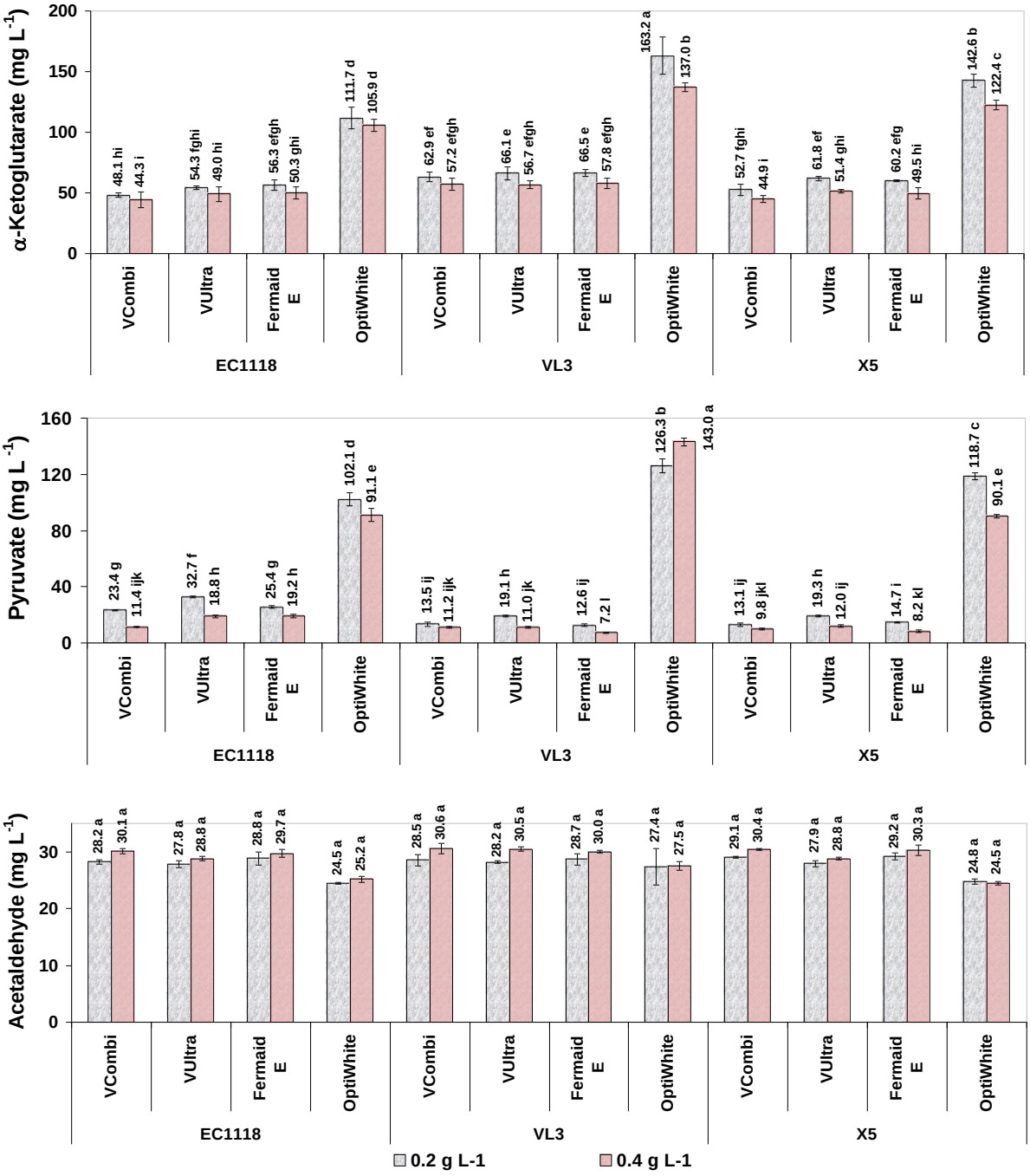


Figure 4-50 Concentration of SO_2-binding compounds present in finished YPF wines produced by three different yeast strains with four nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each compound, whereas those followed by the same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

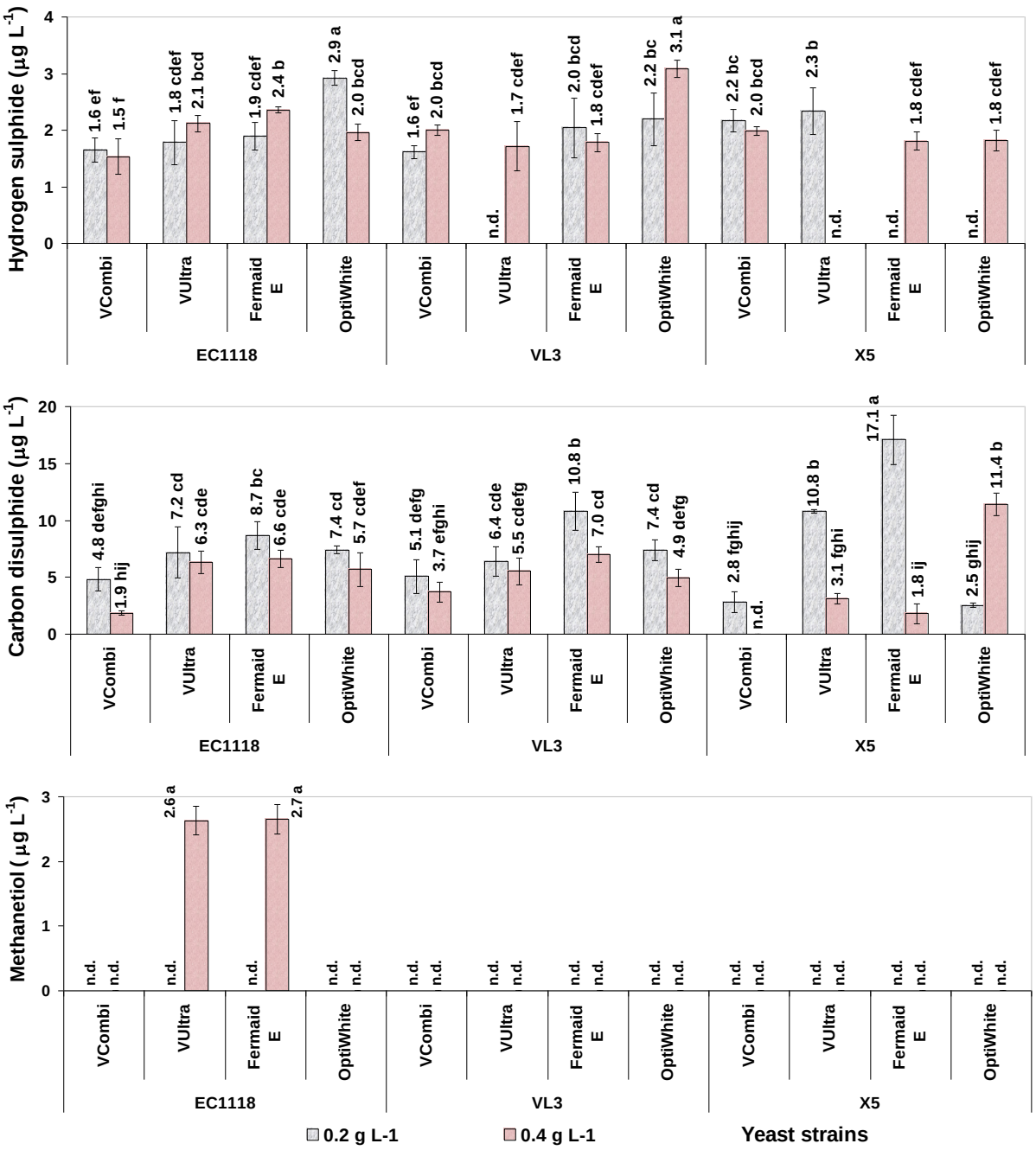


Figure 4-51 Concentration of hydrogen sulphide, carbon disulphide and methanethiol detected in finished YPF wines produced by three different yeast strains with four nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each compound, whereas those followed by the same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

n.d. denotes not detected

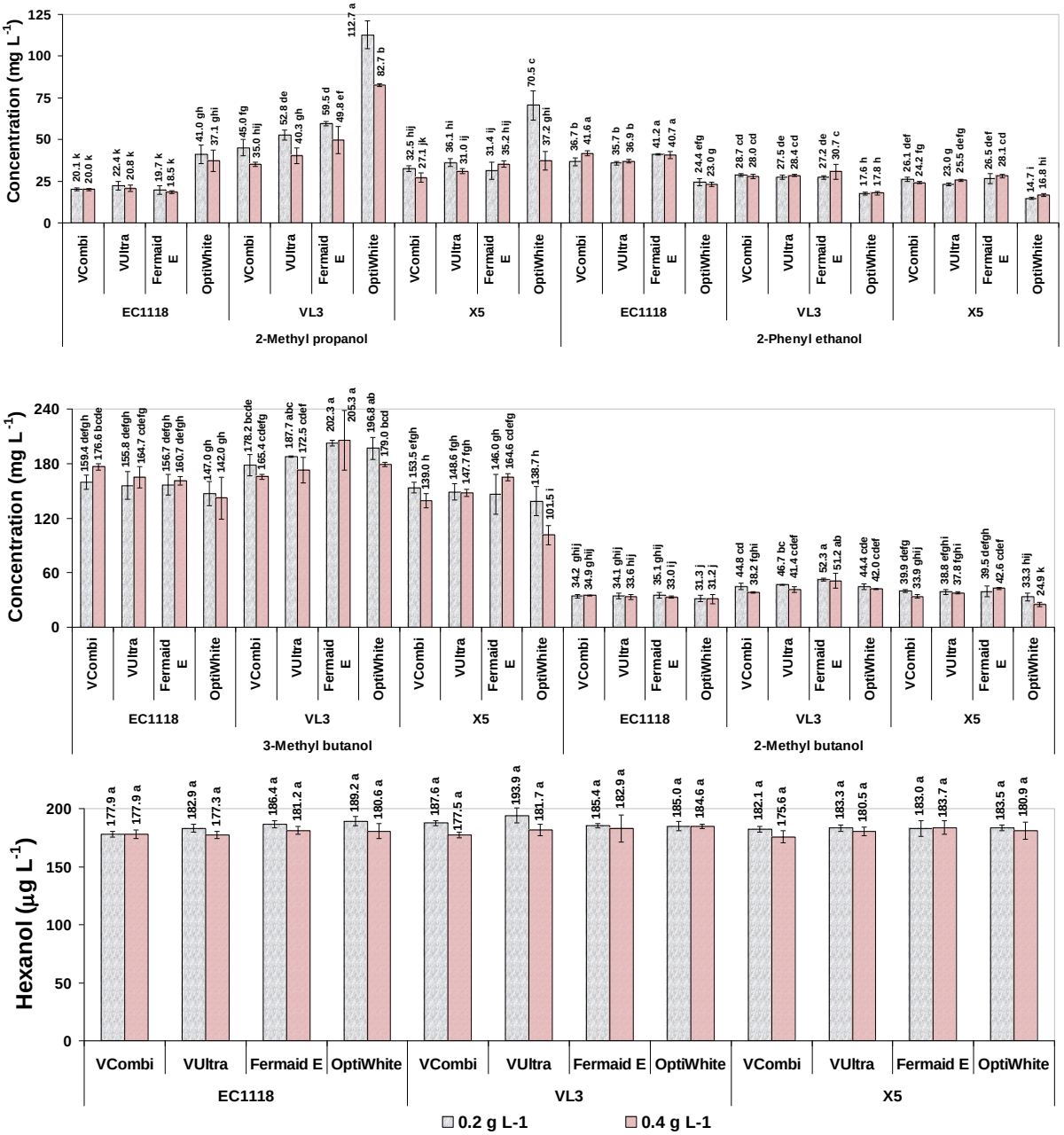


Figure 4-52 Concentration of higher alcohols present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) between treatment combinations of each compound, whereas those followed by the same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

Acetic acid esters: The results demonstrate that these compounds were dependent upon yeast strain, nutrient source and concentration (**Figure 4-53**). It is similar to previous trials of YPF fermentations, concentrations of acetic acid ethyl ester were only detected in small quantities and out of the limit of quantification (50 mg L^{-1}), thus it was present as relative peak area. The addition of OptiWhite at both levels stimulated the highest production of acetic acid ethyl ester by strain VL3 (17.0 $\pm$ 1.3 and 15.3 $\pm$ 0.7 relative peak area, respectively), followed by the low Fermaid E treatment, and by strain X5 at high level of OptiWhite (13.7 $\pm$ 0.8 and 13.6 $\pm$ 2.0 relative peak area, respectively). The greatest amount of acetic acid 2-phenyl ethyl ester was produced by the EC1118 strain in the presence of VCombi at high level (145.0 $\pm$ 11.9 $\mu g\ L^{-1}$), followed by the VUltra and Fermaid E treatments at high level (101.2 $\pm$ 4.9 and 101.5 $\pm$ 6.6 $\mu g\ L^{-1}$, respectively). A high level of VCombi stimulated the highest production of acetic acid 3-methylbutyl ester and acetic acid 2-methylbutyl ester by strain EC1118 (672.4 $\pm$ 70.9 and 43.3 $\pm$ 0.9 $\mu g\ L^{-1}$, respectively), followed by strain VL3 at high level of VCombi (519.8 $\pm$ 53.9 and 32.9 $\pm$ 3.6 $\mu g\ L^{-1}$, respectively) and VUltra treatments (558.4 $\pm$ 45.9 and 35.5 $\pm$ 2.6 $\mu g\ L^{-1}$, respectively).

Ethyl and diethyl esters: As shown in **Table 4-31** and **Table 4-32**, three yeast strains significantly produced different ethyl esters concentrations in response to nutrient supplementation. Succinic acid diethyl ester was detected only in trace amounts in all YPF wines. The addition of VCombi at high level promoted the highest formation of BuEtE by strain EC1118 (259.4 $\pm$ 6.9 $\mu g\ L^{-1}$) followed by strain X5 (196.3 $\pm$ 5.1 $\mu g\ L^{-1}$). These two strains also produced high amounts of this ethyl ester in the high VUltra sample (180.9 $\pm$ 16.1 and 184.4 $\pm$ 2.0 $\mu g\ L^{-1}$, respectively). Supplementation of YPF juice with high OptiWhite level promoted the highest formation of iBuEtE and LAEtE by the VL3 strain (53.4 $\pm$ 12.4 and 13.0 $\pm$ 0.5 $\mu g\ L^{-1}$, respectively) followed by X5 strain (31.6 $\pm$ 4.4 and 12.1 $\pm$ 0.6 $\mu g\ L^{-1}$, respectively). The greatest concentration of PrEtE was produced by strain VL3 in the high OptiWhite sample (143.4 $\pm$ 3.9 $\mu g\ L^{-1}$) and by the X5 strain in the low Fermaid E treatment (138.9 $\pm$ 19.6 $\mu g\ L^{-1}$). In most cases, high level of VCombi addition seemed to decrease the production of iBuEtE, PrEtE and LAEtE, but to increase BuEtE formation.

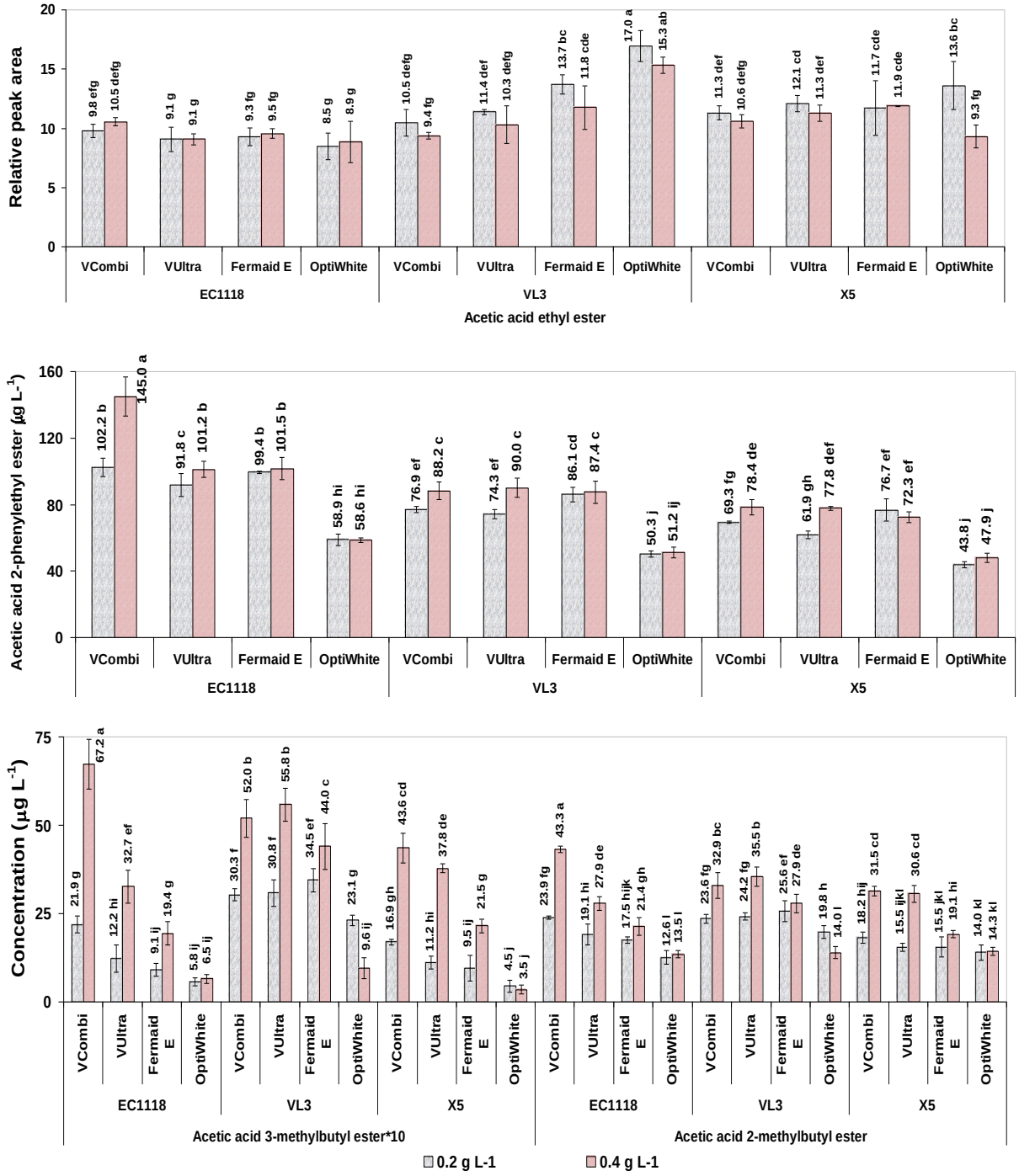


Figure 4-53 Concentration of acetic acid esters present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations (*10 = concentration of compound times 10)

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

Relative peak area = (peak area of sample)/(Peak area of internal standard)

Table 4-31 Concentration of butanoic and isobutanoic acid ethyl esters present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations (g L^{-1})	BuEtE (μg L^{-1})	iBuEtE (μg L^{-1})
EC1118	VCombi	0.2	169.9 ± 1.7 cd	4.2 ± 0.6 g
		0.4	259.4 ± 6.9 a	3.5 ± 0.3 g
	VUltra	0.2	127.5 ± 21.1 gh	5.4 ± 0.8 fg
		0.4	180.9 ± 16.1 bc	3.6 ± 0.9 g
	Fermaid E	0.2	116.1 ± 10.6 hi	6.1 ± 0.3 fg
		0.4	156.4 ± 12.2 de	5.7 ± 1.0 fg
	OptiWhite	0.2	62.1 ± 5.5 k	14.3 ± 1.3 de
		0.4	59.3 ± 8.0 kl	12.3 ± 1.6 e
VL3	VCombi	0.2	104.2 ± 9.0 ij	14.4 ± 2.9 de
		0.4	160.1 ± 13.6 de	13.2 ± 1.3 de
	VUltra	0.2	103.9 ± 18.6 ij	16.5 ± 1.0 cde
		0.4	158.8 ± 8.7 de	13.9 ± 2.2 de
	Fermaid E	0.2	87.1 ± 7.9 j	21.7 ± 2.5 c
		0.4	129.2 ± 14.9 fgh	19.4 ± 3.5 cd
	OptiWhite	0.2	53.0 ± 10.3 kl	53.4 ± 12.4 a
		0.4	42.2 ± 3.0 l	27.1 ± 1.7 b
X5	VCombi	0.2	143.9 ± 2.3 efg	11.6 ± 1.5 ef
		0.4	196.3 ± 5.1 b	11.0 ± 0.5 ef
	VUltra	0.2	118.7 ± 4.7 hi	13.9 ± 1.3 de
		0.4	184.4 ± 2.0 bc	10.4 ± 1.2 ef
	Fermaid E	0.2	105.6 ± 15.0 ij	13.0 ± 2.3 de
		0.4	147.4 ± 9.9 ef	14.6 ± 0.4 de
	OptiWhite	0.2	59.6 ± 6.4 kl	31.6 ± 4.4 b
		0.4	49.3 ± 2.0 kl	20.6 ± 1.6 c

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Table 4-32 Concentration of ethyl esters and diethyl ester present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	PrEtE ($\mu g\ L^{-1}$)	LAEtE ($mg\ L^{-1}$)	SAdiEtE ($\mu g\ L^{-1}$)
EC1118	VCombi	0.2	66.4 ± 2.5 jkl	8.3 ± 0.2 h	Trace
		0.4	53.9 ± 0.8 l	9.4 ± 0.2 efg	Trace
	VUltra	0.2	71.7 ± 2.6 ijkl	8.5 ± 0.1 gh	Trace
		0.4	59.6 ± 6.5 kl	9.0 ± 0.1 fg	Trace
	Fermaid E	0.2	73.5 ± 8.1 hijk	8.3 ± 0.1 h	Trace
		0.4	66.5 ± 1.9 jkl	8.9 ± 0.2 fg	Trace
	OptiWhite	0.2	81.4 ± 12.7 ghij	10.1 ± 0.3 cde	Trace
		0.4	85.8 ± 18.5 fghi	9.5 ± 0.2 def	Trace
VL3	VCombi	0.2	90.9 ± 16.8 fgh	9.0 ± 0.2 fg	Trace
		0.4	65.4 ± 8.4 jkl	9.6 ± 0.3 efg	Trace
	VUltra	0.2	95.1 ± 14.4 efg	9.2 ± 0.1 fg	Trace
		0.4	69.7 ± 9.5 ijkl	9.6 ± 0.3 efg	Trace
	Fermaid E	0.2	126.4 ± 7.6 bc	9.1 ± 0.2 fg	Trace
		0.4	102.4 ± 15.9 def	9.8 ± 0.7 cde	Trace
	OptiWhite	0.2	123.0 ± 5.6 bc	13.0 ± 0.5 a	Trace
		0.4	143.4 ± 3.9 a	10.4 ± 0.5 cd	Trace
X5	VCombi	0.2	109.5 ± 4.9 cde	9.0 ± 0.2 fg	Trace
		0.4	80.6 ± 3.5 ghij	9.7 ± 0.3 def	Trace
	VUltra	0.2	120.9 ± 6.7 c	9.3 ± 0.3 fg	Trace
		0.4	93.7 ± 2.1 efg	9.6 ± 0.3 def	Trace
	Fermaid E	0.2	138.9 ± 19.6 ab	8.8 ± 0.4 gh	Trace
		0.4	125.7 ± 4.1 bc	9.7 ± 0.5 def	Trace
	OptiWhite	0.2	118.0 ± 13.2 cd	12.1 ± 0.6 b	Trace
		0.4	102.6 ± 11.5 def	10.7 ± 0.2 c	Trace

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Medium-chain fatty acids and related ethyl esters: As given in **Table 4-33** and **Table 4-34**, the three yeast strains developed significant differences of these volatile compounds in response to nutrient addition. Strain EC1118 developed the greatest concentration of hexanoic, octanoic and decanoic acids in the high VCombi sample (5.8 ± 0.4, 4.9 ± 0.6 and 0.37 ± 0.07 mg L^{-1}, respectively), followed by the high VUltra treatment (4.9 ± 0.3, 4.0 ± 0.3, 0.30 ± 0.02 mg L^{-1}, respectively) and the VCombi sample (4.7 ± 0.1, 3.7 ± 0.3, 0.30 ± 0.14 mg L^{-1}, respectively). It also produced the greatest amounts of HAEtE, OAEtE and DAEtE in the high VCombi treatment (830.5 ± 67.0, 1101.9 ± 46.0 and 324.2 ± 15.5 μg L^{-1}, respectively) and followed by the high VUltra sample (633.8 ± 65.4, 872.9 ± 62.9, 268.6 ± 14.3 μg L^{-1}, respectively). A high concentration of VCombi also promoted high formation of OAEtE and DAEtE by the X5 strain (891.6 ± 30.1 and 294.4 ± 16.1 μg L^{-1}, respectively). Overall, strain EC1118 seemed to be the highest producer of MCFAs and MCFA ethyl esters in certain nutrient condition, while the VL3 strain was the lowest producer but this varied depending on nutrient source and concentration.

Monoterpenes: The results show that three yeast strains liberated similar concentrations of α-terpineol ranging from 9.4 ± 0.4 to 10.6 ± 0.2 μg L^{-1} (**Figure 4-54**). Linalool, *trans*-linalool oxide and *cis*-linalool oxide were not detected in all YPF wines.

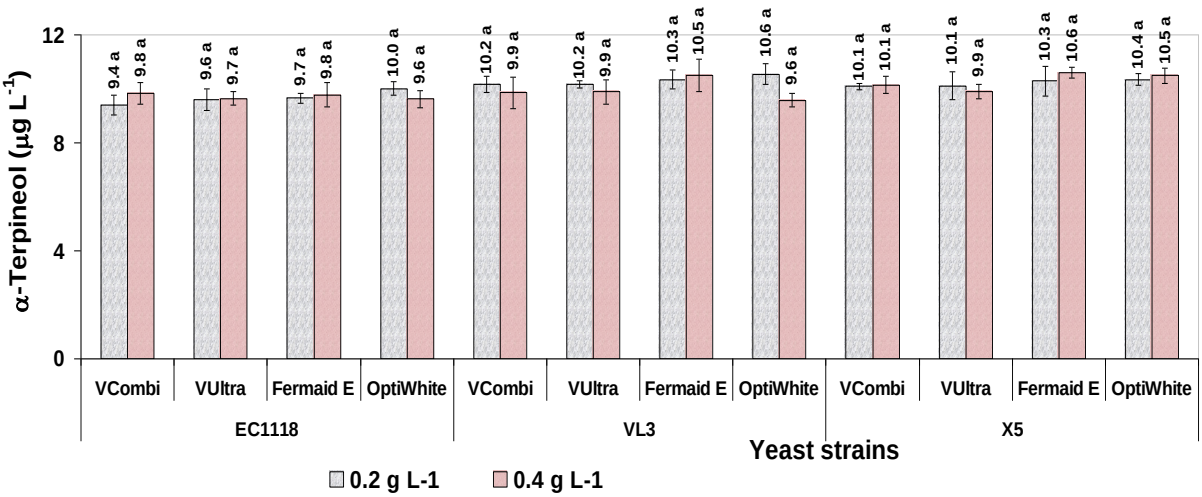


Figure 4-54 Concentration of α-terpineol present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Vertical bars represent standard deviations from three fermentation replicates. Means followed by the same letters on the top of the bar are not significantly different ($p>0.05$) between treatment combinations according to the DMRT test.

Table 4-33 Concentration of fatty acids present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	Hexanoic acid ($mg\ L^{-1}$)	Octanoic acid ($mg\ L^{-1}$)	Decanoic acid ($mg\ L^{-1}$)
EC1118	VCombi	0.2	4.7 ± 0.1 b	3.7 ± 0.3 bc	0.30 ± 0.14 b
		0.4	5.8 ± 0.4 a	4.9 ± 0.6 a	0.37 ± 0.07 a
	VUltra	0.2	4.2 ± 0.4 cd	3.2 ± 0.5 bcd	0.23 ± 0.04 cd
		0.4	4.9 ± 0.3 b	4.0 ± 0.3 b	0.30 ± 0.02 b
	Fermaid E	0.2	4.1 ± 0.2 d	3.0 ± 0.2 cde	0.20 ± 0.01 de
		0.4	4.5 ± 0.3 bc	3.5 ± 0.4 bc	0.27 ± 0.02 bc
	OptiWhite	0.2	3.3 ± 0.1 e	2.2 ± 0.2 fg	0.17 ± 0.02 e
		0.4	2.8 ± 0.5 ef	1.7 ± 0.5 gh	0.10 ± 0.04 f
VL3	VCombi	0.2	3.2 ± 0.2 e	1.9 ± 0.2 fg	0.10 ± 0.01 f
		0.4	3.8 ± 0.3 d	2.8 ± 0.3 cde	0.20 ± 0.02 de
	VUltra	0.2	3.2 ± 0.3 e	1.9 ± 0.3 fg	0.10 ± 0.01 f
		0.4	3.8 ± 0.3 d	2.6 ± 0.3 def	0.20 ± 0.01 de
	Fermaid E	0.2	2.7 ± 0.1 e	1.5 ± 0.1 gh	0.10 ± 0.01 f
		0.4	3.0 ± 0.1 e	1.8 ± 0.2 fg	0.10 ± 0.01 f
	OptiWhite	0.2	2.5 ± 0.2 f	1.3 ± 0.1 h	0.10 ± 0.01 f
		0.4	2.4 ± 0.1 f	1.3 ± 0.0 h	0.10 ± 0.01 f
X5	VCombi	0.2	3.7 ± 0.1 d	2.6 ± 0.2 def	0.10 ± 0.01 f
		0.4	4.5 ± 0.2 cd	3.5 ± 0.2 bcd	0.20 ± 0.01 de
	VUltra	0.2	3.2 ± 0.2 e	2.1 ± 0.3 fg	0.17 ± 0.03 e
		0.4	4.2 ± 0.1 d	3.1 ± 0.3 cde	0.20 ± 0.02 de
	Fermaid E	0.2	3.2 ± 0.0 e	2.1 ± 0.1 fg	0.17 ± 0.01 e
		0.4	3.6 ± 0.1 d	2.4 ± 0.1 efg	0.20 ± 0.02 de
	OptiWhite	0.2	2.8 ± 0.1 e	1.6 ± 0.1 fg	0.10 ± 0.01 f
		0.4	2.9 ± 0.3 e	1.7 ± 0.3 gh	0.10 ± 0.02 f

Each value shows the mean ± standard deviation from three fermentation replicates.

Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

Table 4-34 Concentration of fatty acid ethyl esters present in finished YPF wines developed by three different yeast strains with four nutrient sources at two different concentrations

Yeasts	Nutrients	Concentrations ($g\ L^{-1}$)	HAEtE ($\mu g\ L^{-1}$)	OAEtE ($\mu g\ L^{-1}$)	DAEtE ($\mu g\ L^{-1}$)
EC1118	VCombi	0.2	664.5 ± 31.5 b	859.6 ± 34.2 b	253.6 ± 2.8 cd
		0.4	830.5 ± 67.0 a	1101.9 ± 46.0 a	324.2 ± 15.5 a
	VUltra	0.2	519.9 ± 91.1 de	684.6 ± 115.2 de	201.2 ± 31.1 efg
		0.4	633.8 ± 65.4 bc	872.9 ± 62.9 b	268.6 ± 14.3 bc
	Fermaid E	0.2	502.8 ± 42.0 e	634.6 ± 23.8 ef	202.2 ± 7.2 efg
		0.4	577.1 ± 47.0 cd	758.2 ± 49.2 cd	234.3 ± 9.1 de
	OptiWhite	0.2	283.4 ± 6.9 ghi	340.1 ± 23.7 ij	111.6 ± 11.6 jk
		0.4	273.6 ± 12.2 hi	343.7 ± 25.7 ij	108.0 ± 26.6 jk
VL3	VCombi	0.2	208.3 ± 36.5 ijk	404.5 ± 59.1 hi	130.2 ± 20.3 ij
		0.4	363.5 ± 15.7 f	707.8 ± 67.2 de	234.8 ± 13.5 de
	VUltra	0.2	221.0 ± 67.9 ij	394.3 ± 53.2 hi	131.1 ± 31.2 ij
		0.4	352.6 ± 19.9 fg	669.4 ± 22.7 de	204.0 ± 5.6 efg
	Fermaid E	0.2	146.8 ± 22.8 jk	286.6 ± 38.8 jk	96.6 ± 10.3 kl
		0.4	239.7 ± 32.7 i	473.6 ± 54.6 gh	135.2 ± 8.4 ij
	OptiWhite	0.2	71.7 ± 17.2 l	133.9 ± 34.6 lm	67.6 ± 7.5 l
		0.4	54.7 ± 11.9 k	101.6 ± 14.4 m	71.7 ± 1.7 l
X5	VCombi	0.2	391.4 ± 15.1 f	673.0 ± 40.4 de	212.0 ± 22.0 ef
		0.4	497.9 ± 24.7 e	891.6 ± 30.1 b	294.4 ± 16.1 ab
	VUltra	0.2	323.6 ± 36.2 fgh	555.5 ± 40.6 fg	181.1 ± 13.6 fgh
		0.4	469.5 ± 12.4 e	808.6 ± 12.2 bc	254.4 ± 3.9 cd
	Fermaid E	0.2	280.4 ± 34.7 ghi	455.6 ±46.0 h	152.2 ± 8.6 hi
		0.4	330.0 ± 56.0 fgh	641.2 ± 52.0 ef	172.8 ± 49.1 gh
	OptiWhite	0.2	147.4 ± 21.0 k	199.1 ± 47.3 kl	106.1 ± 16.8 jk
		0.4	148.9 ± 5.0 k	219.3 ± 29.3 kl	84.3 ± 12.9 kl

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters within the same column are significantly different ($p<0.05$) among treatment combinations according to the DMRT test.

4.4 Volatile thiols involved in varietal aromas of yellow passion fruit wines

The volatile thiols involved in varietal aromas detected in YPF wines were selected from the trials described in the chapters of **4.3.3** and **4.3.4**. These selected YPF wines had desirable fermentation characteristics and wine derived fermentation aromas with low undesirable metabolites. The volatile thiols detected in YPF wine obtained from chapter **4.3.3** are given in **Figure 4-55** to **4-56**, while those detected in YPF wines obtained from chapter **4.3.4** are shown in **Figure 4-57**.

Figure 4-55 shows that the 3-sulphanylhexanol (3SH) was identified in the prepared YPF juice (498 ng L^{-1}), while acetic acid 3-sulphanylhexyl ester (3SHA) was present in trace quantities (data not shown). Commercial *Saccharomyces* yeast strains had a significant impact on the production of 3SH and its ester (3SHA) in YPF wines. The commercial yeasts released approximately 7.1-fold to 11.2-fold of 3SH from its precursor in YPF juice but its concentrations depended on the yeast strain. At 0.5 g L^{-1} VCombi addition, strain EC1118 significantly appeared to be the most effective producer of 3SH and 3SHA in YPF wine (5594.0 $\pm$ 1060.5 and 502.1 $\pm$ 84.8 ng L^{-1}, respectively). The LittoLevure strain followed a similar pattern of the high 3SH liberation (4802.7 $\pm$ 163.0 ng L^{-1}) as strain EC1118, but showed less 3SHA production. Strain VL3 seemed to be the least efficient in 3SH aroma release in the YPF wine.

The EC1118 strain with the addition of 0.25 g L^{-1} DAP produced the greatest concentration of 3SH and 3SHA in the YPF wines (8317.8 $\pm$ 2384.1 and 470.3 $\pm$ 194.2 ng L^{-1}, respectively) as demonstrated in **Figure 4-56**. The addition of 0.5 g L^{-1} VCombi also promoted strain EC1118 to produce the highest amount of both volatile thiols in the YPF wines (5594.0 $\pm$ 1060.5 and 502.1 $\pm$ 84.8 ng L^{-1}, respectively). These volatile thiols seemed to be least obvious in the YPF wine produced by strain X5 with the 0.25 g L^{-1} VCombi supplementation.

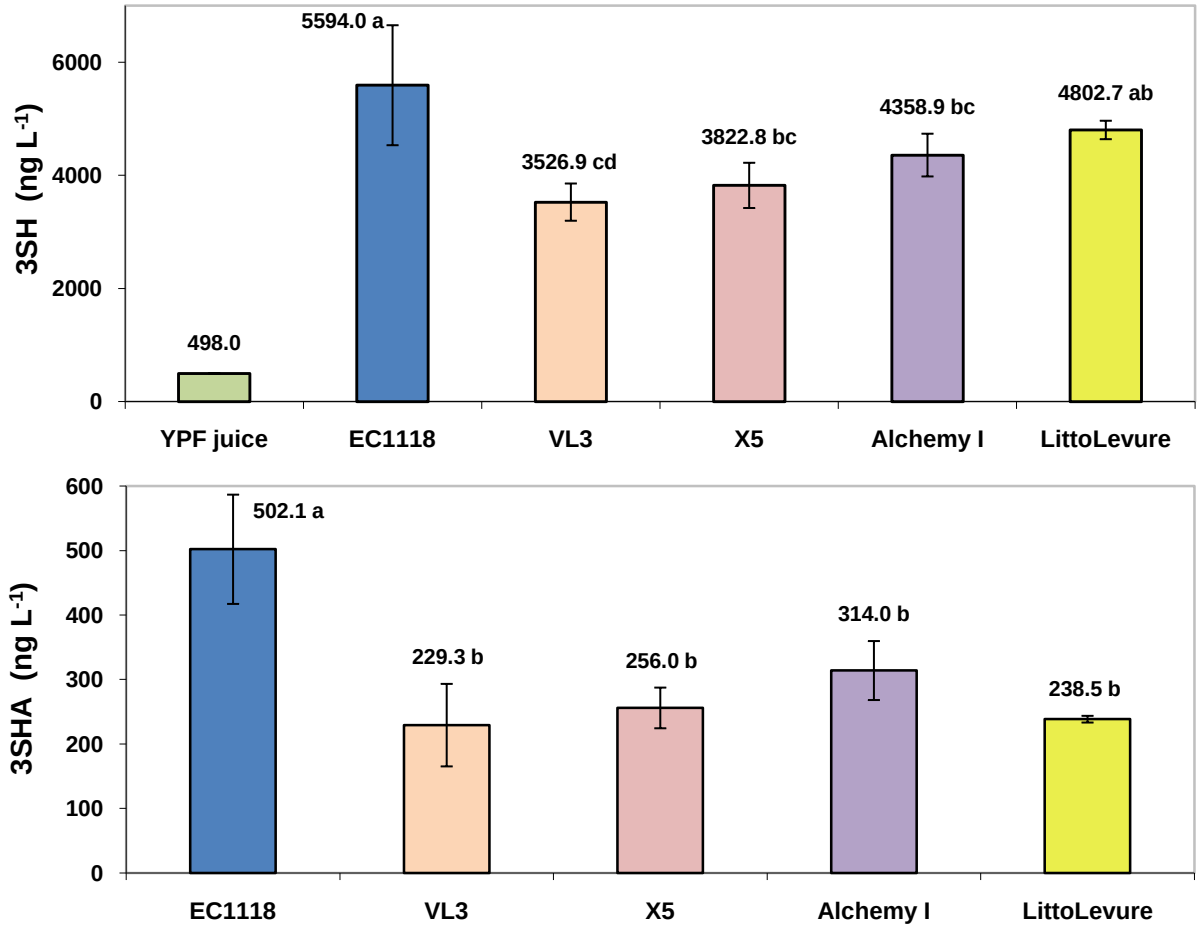


Figure 4-55 Concentration of 3SH and 3SHA detected in prepared YPF juice and finished YPF wines produced by five different yeast strains with the addition of VCombi at the high level

Vertical bars represent standard deviations from three fermentation replicates, except YPF juice obtained from two sample replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains according to the DMRT test.

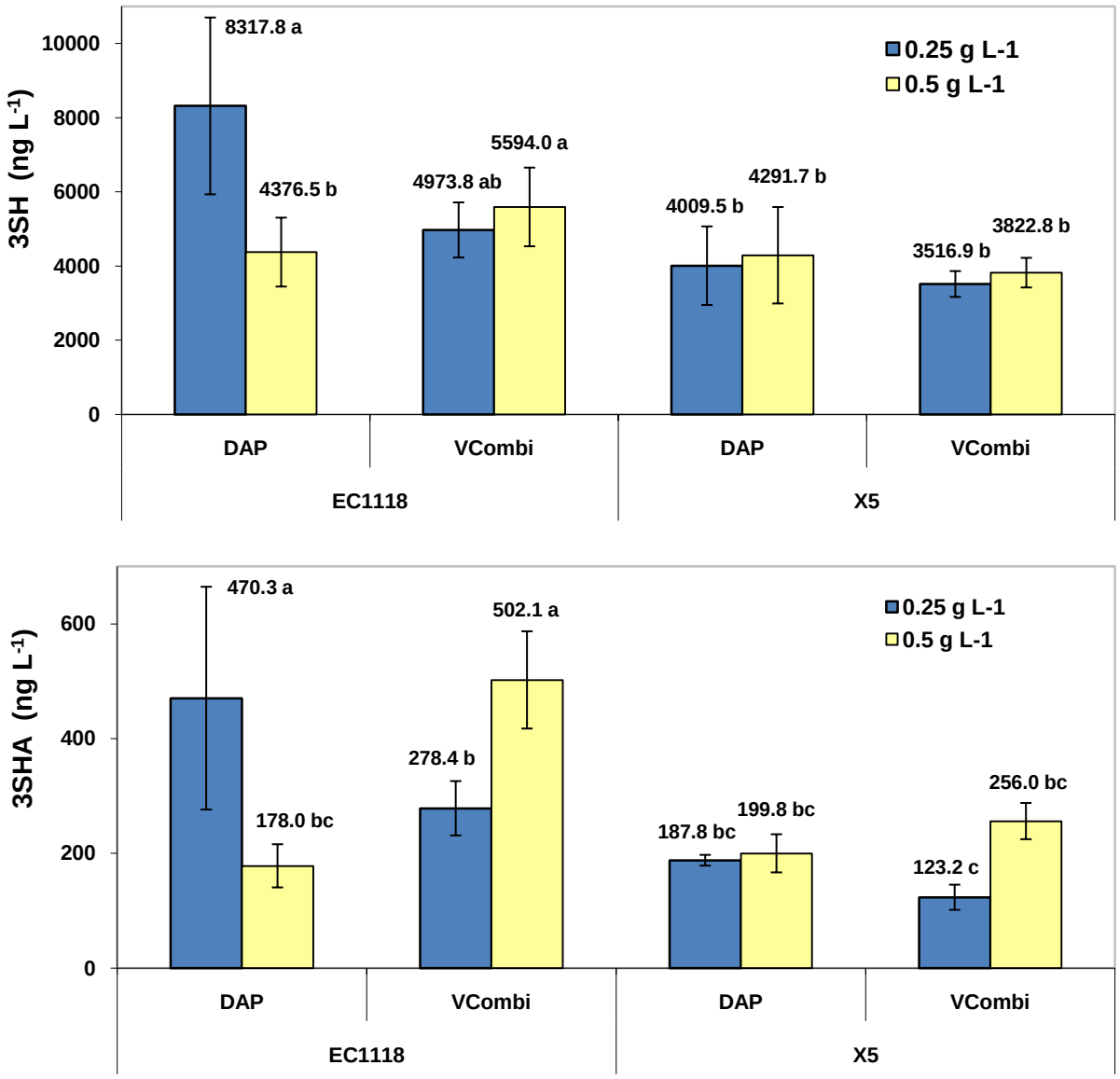


Figure 4-56 Concentration of 3SH and 3SHA detected in YPF wines produced by two yeast strains with the addition of two nutrient sources at two levels

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains according to the DMRT test.

The analysis of the prepared YPF juice for trial **3.9.4** clearly indicated that the 426 ng L^{-1} 3SH and trace amounts of 3SHA were present as well (data not shown). The *Saccharomyces* yeast strains had significantly the greater impact on the liberation of 3SH in YPF wines than the nutrient supplementations. The X5 strain liberated a higher concentration of 3SH in the YPF wine with either VCombi or VUltra supplementation (5527.5 $\pm$ 639.5 and 5206.1 $\pm$ 397.1 ng L^{-1}, respectively) than strain EC1118. Both factors of the yeast strain and the

nutrient source had no influence on the 3SHA production ranging from 263.8 ± 73.5 to 333.9 ± 47.5 ng L^{-1} (**Figure 4-57**). Nevertheless, 3SHA amounts in YPF wine tended to be higher in the VCombi treatment.

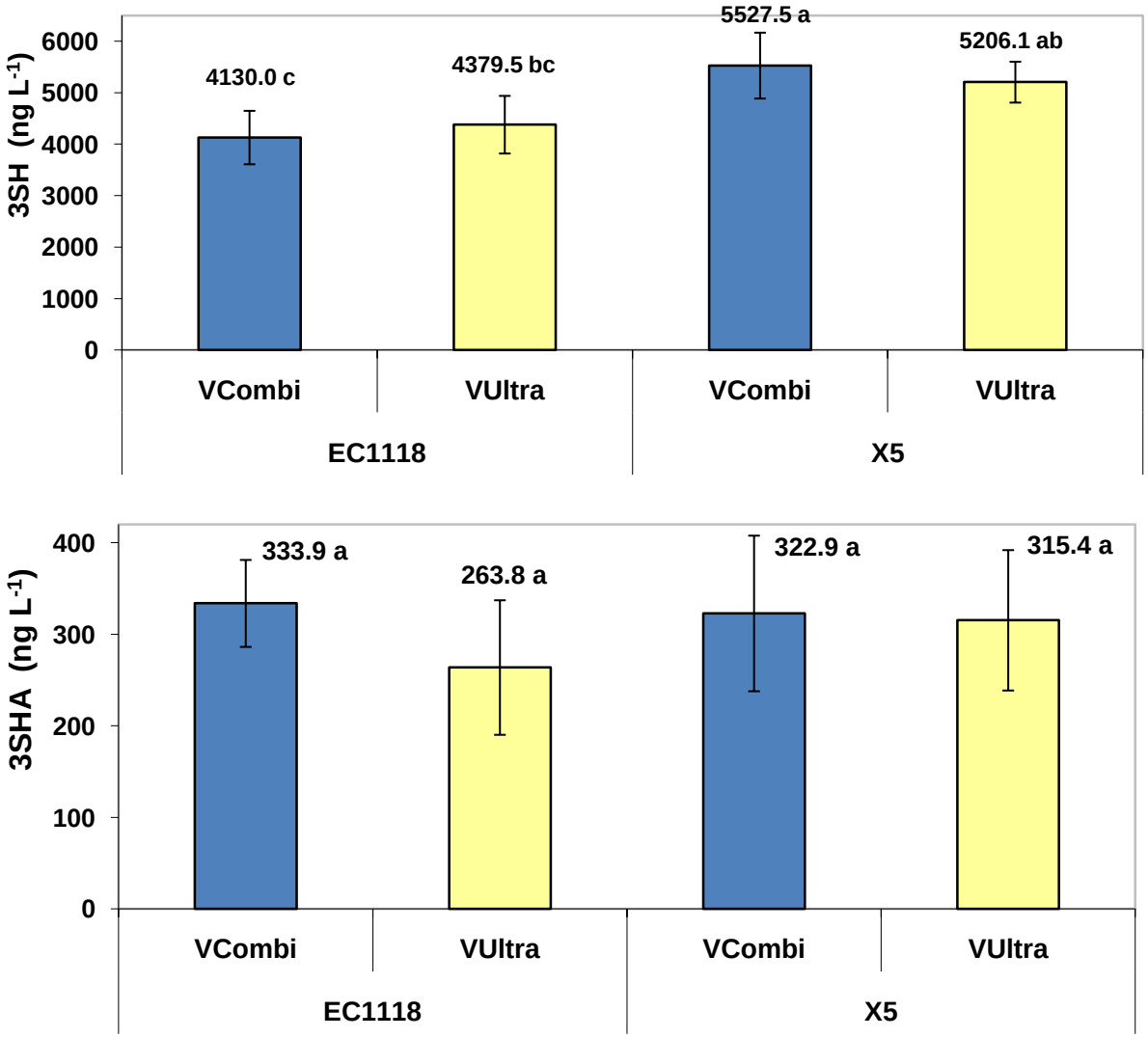


Figure 4-57 Concentration of 3SH and 3SHA detected in finished YPF wines produced by two different yeast strains with the addition of VCombi and VUltra at 0.4 g L^{-1}

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations, whereas those followed by the same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

5. DISCUSSION

This study is focused on the improvement of the fermentation characteristics, varietal and important aromas in grape wines as well as in yellow passion fruit (YPF) wine by optimal choice of commercial *Saccharomyces* yeast strains and nutrient supplementations; nutrient sources/compositions and concentrations.
The obtained results from the investigated fermentation experiments of Scheurebe and Sauvignon blanc grape wines (chapter **5.1** and **5.2**) and YPF wines (chapter **5.3**) are discussed in the following chapters (see experimental outline for all fermentation trials in **Figure A-2**).

5.1 Effects of commercial *Saccharomyces* yeast strains and nutrient supplements on wine fermentative characteristics and quality of Scheurebe grape wines

The experimental discussions, which are given in the following chapters, comprise five Scheurebe grape juice fermentation trials that investigated the effect of commercial *Saccharomyces* yeast strains and nutrient supplementation; nutrient source/composition and concentrations, on fermentation kinetics, some metabolic compounds and volatile compounds of finished wines (chapter **5.1.1** to **5.1.2**).

5.1.1 Effect of commercial *Saccharomyces* yeast strains and nutrient sources on fermentative characteristics, metabolic compounds and wine aroma of Scheurebe grape wines

Strain X5 had a longer fermentation time than strain EC1118 in the OptiWhite and control fermentation, but the addition of Fermaid E reduced fermentation time (**Figure 4-1**). Nevertheless, all treatments completed fermentation after 11-14 days with low residual sugar less than 2 g L^{-1} (**Figure 4-2**). It has been reported that nutrient composition in complex mixtures affects yeast growth rate and favours higher rates than single compounds. Thus, mixtures of amino acids and vitamins give higher growth rates than the most preferred single nitrogen sources (Henschke & Jiranek, 1993; Albers et al., 1996; ter Schure et al., 2000; da Cruz et al., 2002; Beltran et al., 2004, 2005; Ribéreau-Gayon et al., 2006 a). It is linked to the ability of yeast to directly incorporate amino acids into protein, thereby minimising the need to maintain an energetically expensive amino acid synthetic capability (Bell & Henschke, 2005). In addition, nitrogen metabolism is central to cell growth and regulates other pathways, including sugar metabolism in yeast (Bell & Henschke, 2005; Ugliano et al., 2007).

Both yeast strain and nutrient source did not affect the formation of glycerol and acetaldehyde (**Figure 4-2** and **4-3**), which were within the normal ranges of concentration in wines at 4-15 g L^{-1} and 75-100 mg L^{-1}, respectively (Rankine & Bridson, 1971; Schreier, 1979; Ough & Amerine, 1988; Swiegers et al., 2005 a; Ribéreau-Gayon et al., 2006 b).
Strain EC1118 produced high amounts of acetic acid in the control and OptiWhite treatments, while strain X5 formed high levels in the control and DAP fermentations (**Table 4-1**). According to Bely et al. (2003), increasing nitrogen availability increases NADH production, which in turn reduces the need of the yeast cell to produce acetic acid to control redox balance. On the contrary, a high production of acetic acid by strain X5 in a high-nitrogen juice, as DAP might be related at least in part to a redox balancing reaction in response to high glycerol production, which generates a net deficit of NADH (Vilanova et al., 2007). Nonetheless, its amounts in these wines were within the normal range at below 0.7 g L^{-1} (Corison et al., 1979; Ribéreau-Gayon et al., 2006 b). In addition, they did not undergo malolactic fermentation (Ough & Amerine, 1988; Radler, 1993). Concentrations of the other acids were similar among the treatment combinations and within the range of the reported wine values above.

As shown in **Figure 4-3**, it is likely that nitrogen in grape juice, including control, was sufficient for yeast metabolism of the two yeast strains as concentrations of SO_2-binding compounds in wines were less than 100 mg L^{-1} (Rankine, 1968 a, 1968 b; Radler, 1993; Ribéreau-Gayon et al., 2006 a). In addition, the supplementation of Fermaid E, which contains thiamine, significantly reduced both keto acids. In accordance with some studies, thiamine effectively reduces keto acid concentrations by the enzymatic decarboxylation (Delfini et al., 1980; Delfini & Vormica, 2001; Ribéreau-Gayon et al., 2006 a; Jackson, 2008).
Both yeast strain and nutrient source had no influence on the production of dimethyl sulphide (**Figure 4-4**) and its levels in most wines were below the aroma threshold of 25 μg L^{-1} (De Mora et al., 1986; Rauhut, 1993; Mestres et al., 2000; Swiegers et al., 2005 a). Although the X5 strain produced H_2S in the final wine, its formation decreased with the addition of Fermaid E, DAP and OptiWhite. In addition, its levels were below the threshold value (50-80 μg L^{-1}), which excluded the occurrence of an off-flavour of “rotten egg” (Rauhut, 1996; Bell & Henschke, 2005; Rauhut, 2009). In agreement with several studies, addition of nutrient decreased the formation of H_2S by yeast (Rauhut & Kürbel, 1994; Jiranek et al. 1995 a, 1995 b; Rauhut et al., 1995, 1997, 2000 a, 2000 b; Mendes-Ferreira et al., 2004, 2009).

The formation of higher alcohols, acetic acid esters, volatile fatty acids and their ethyl esters produced by the two yeast strains varied depending on the nutrient sources. Strain EC1118 developed high amounts of 2-methyl propanol and 3-methyl butanol in most nutrient sources

(**Table 4-2**). This might reflect a less efficient usage of nitrogen, resulting in an increase of carbon flux related to branched-chain amino acid metabolism by this strain (Eden et al., 2001; Ribéreau-Gayon et al., 2006 a; Carrau et al., 2008). The relationship between higher alcohol production and high nitrogen availability was in agreement with earlier studies (Oshita et al., 1995; Hernandez-Orte et al., 2005; Vilanova et al., 2007). They suggested that at higher yeast assimilable nitrogen, sufficient nitrogen is available for amino acid biosynthesis, which reduces the surplus of α-keto acids and, hence, the higher alcohols produced. The formation of the other higher alcohols was similar among the treatment combinations (**Table 4-2** and **Figure 4-5**). Interestingly, concentrations of these higher alcohols were below 300 mg L^{-1} that they are usually considered to contribute to a wine's aromatic complexity as previously reported (Lambrechts & Pretorius, 2000; Clarke & Bakker, 2004; Francis & Newton, 2005; Swiegers & Pretorius, 2005; Ribéreau-Gayon et al., 2006 b). In addition, levels of 2-phenyl ethanol, which elicits floral and rose-like aromas, in most wines were above the aroma threshold of 10 mg L^{-1} as reported in literatures above.

The EC1118 strain seemed to be a higher producer of most acetic acid esters, ethyl esters and hexanoic acid ethyl ester (excluding succinic acid diethyl ester) than strain X5 with less response to nutrient sources (**Figure 4-5**, **4-6** and **Table 4-3**, **4-4**), while these volatile esters produced by strain X5 were most evident in the Fermaid E variant. It has been shown that the final concentration of acetic acid esters of higher alcohols is the result of the balance between alcohol acyl transferase enzymes promoting acetic acid ester biosynthesis and esterase enzymes promoting their hydrolysis (Mauricio et al., 1993; Fukuda et al., 1998; Vianna & Ebeler, 2001). Increased nitrogen availability promotes the expression of the *ATF1* and *ATF2* genes, which encode the alcohol acyl transferase enzymes (Yoshimoto et al., 2002). Some studies have also reported that the increase in ester formation is directly related to a high nitrogen level (Rapp & Versini, 1991; Beltran et al., 2005; Hernández-Orte et al., 2006 a). Miller et al. (2007) have suggested that when ammonium was added into the juice, the amino acids were not used for cellular structures and growth but, rather, induced the production of secondary metabolites like volatile esters. In addition, when Superstart was added, high amounts of succinic acid diethyl ester were formed by the two yeast strains. Rosi et al. (2008) have also revealed that organic nitrogen like inactive dry yeast promoted yeast cells to develop high amounts of esters.

From a wine maker's view, the most important esters in wine are acetic acid ethyl ester, acetic acid 2-phenyl ethyl ester, acetic acid 3-methylbutyl ester and ethyl esters of medium-chain fatty acids (Guth, 1997 b; Francis & Newton, 2005; Ribéreau-Gayon et al., 2006 b; Sumby et al., 2009). They play a major role in the fruity and floral aroma of young wines and also positively influence the general quality of the wine. They were prominent in most

EC1118 wines as well as the X5 wine with Fermaid E addition and their levels were above the thresholds as reported in literatures above. On the contrary, the concentrations of lactic acid ethyl ester in most wines were below the threshold at 50-200 μg L^{-1}, which imparts milk, buttery and soapy aromas and mainly increases during malolactic fermentation.

These findings pointed out that the two yeast strains and nutrient sources did not affect the concentrations of most monoterpenes; *trans*-linalool oxide, *cis*-linalool oxide, linalool and α-terpineol detected in wines (**Figure 4-7**). Their concentrations in the wine treatments were below their aroma threshold that contributes to varietal aroma (Guth, 1997 b; Ribéreau-Gayon et al., 2006 b; Sumby et al., 2009). Großmann et al. (1990) suggested that under normal wine making conditions, endogenous β-glycosidases have a limited effect on the development of terpenes, because their activity reaches an optimum at pH 5 and the clarification of juice can also inhibit their activity.

5.1.2 Effect of thirteen commercial *Saccharomyces* yeast strains on fermentative characteristics, metabolic compounds and wine aroma of Scheurebe grape wines

The fermentations of fresh Scheurebe grape juices with thirteen commercial *Saccharomyces* yeast strains; EC1118, Sauvignon, VL3, X5, X16, VIN13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2 and QA23 had different fermentation kinetics and chemical composition. As demonstrated in **Figure 4-8** and **4-9**, strains EC1118, VIN13 and AWRI R2 had the fastest fermentation kinetics and times with low residual sugar (below 2 g L^{-1}) but high glycerol levels. On the contrary, the Sauvignon, Alchemy I, 4F9, LVCB and LittoLevure strains resulted in sluggish or stuck fermentations with high residual sugar (above 10 g L^{-1}) but low glycerol amounts. It is likely that they had extremely higher nutrient demands than the other strains as the grape juice was not supplemented with any kind of nutrients, although it had high initial nitrogen; 0.09 g L^{-1} ammonium, 194 mg L^{-1} free amino nitrogen (as shown in **3.7.2**) and 1314.6 mg L^{-1} total amino acid content without proline (**Table A-1**). It was exceptional for the Alchemy II and QA23 strains that they had sluggish and long fermentation durations, but finished fermentations with low residual sugar. A sluggish or stuck fermentation can be due to nitrogen deficiency, thiamine depletion of the must, CO_2 and ethanol production (Bely et al., 1990; Bisson, 1999, 2000; Julien et al., 2000; Blateyron & Sablayrolles, 2001). In addition, a low or lack initial level of nitrogen acts by limiting growth rate and biomass formation of yeast which may result in a low rate of sugar catabolism and decrease in sugar transport activity by yeast (Salmon, 1989; Bely et al., 1991; Monteiro & Bisson, 1991; Salmon, 1996; Alexandre & Charpentier, 1998; Colombié et al., 2005).

Although the yeast strain strongly influenced the glycerol production under controlled conditions as reported in several studies (Radler & Schütz, 1982; Scanes et al., 1998;

Antonelli et al., 1999; Remize et al., 1999, 2001; Reynolds et al., 2001; Erasmus et al., 2004), its concentrations were within the concentrations reported for the wine.

Fermentations with strain EC1118 resulted in the highest amounts of lactic acid and keto acids but the lowest acetic acid and acetaldehyde productions, whereas citric acid was higher in the VL3 and QA23 wines (**Table 4-5** and **Figure 4-10**). Strains VL3, X5, X16, 4F9, LVCB and LittoLevure were the highest α-ketoglutarate and acetaldehyde producers, while strain Sauvignon was the highest producer of acetaldehyde, H_2S and CS_2 (**Figure 4-11**). It is worth noting that the strains with fast fermentation kinetics and high sugar consumption produced the lowest volatile acidity, H_2S and acetaldehyde.

It appears that the ability of a yeast strain to produce H_2S is genetically caused as H_2S production by different wine yeast strains varies under similar conditions (Thornton & Bunker, 1989; Henschke & Jiranek, 1993; Jiranek et al., 1995 b; Spiropoulos et al., 2000). Berry & Watson (1987) proposed that yeasts with low vitality such as in other sluggish or stuck fermentations can tend to autolyse faster and release consequently H_2S (Henschke & Jiranek, 1993). In addition, the ability of yeast strains to produce acetaldehyde is depending on the activity of the enzyme involved in its synthesis like alcohol dehydrogenase (Vannini et al., 1994; Pérez-Coello et al., 1999; Marks et al., 2003). Nevertheless, it could be confirmed that the wine samples had neither malolactic fermentation nor the detectable spoilage of vinegar-like odour and the off-flavour of rotten egg (see reported literatures in **5.1.1**). These compounds had no impact on the quality of the final wines and ranged within the usual values in wines.

The use of *Saccharomyces* yeast strains during fermentation considerably contributes to variations in the production of volatile compounds (Antonelli et al., 1999; Pérez-Coello et al., 1999; Herjavec et al., 2003; Plata et al., 2003; Romano et al., 2003; Swiegers et al., 2005 a; Regodón Mateos et al., 2006; Miller et al., 2007; Jackson, 2008). The greatest producer of higher alcohol and ethyl ester (excluding isobutanoic acid ethyl ester) was strain EC1118 (**Table 4-6**, **4-7** and **Figure 4-12**). Strains QA23, VL3 and X16 also produced similar high amounts of 2-phenyl ethanol (floral and rose-like aromas) at a level above the aroma threshold (Clarke & Bakker, 2004; Francis & Newton, 2005; Swiegers et al., 2005 a; Bartowsky & Pretorius, 2009; Sumby et al., 2009). The levels of hexanol were similar among the thirteen yeast strains. This was supported by Bell & Henschke (2005) who reviewed that hexanol is formed from a must-derived precursor, probably hexanal, during must processing, since yeast are not able to synthesize it. At low concentration below 300 mg L^{-1}, higher alcohols are considered desirable (see previous literatures in **5.1.1**). Interestingly, concentrations of ethyl and diethyl esters in the final wines were above their aroma thresholds, which elicit pleasant floral and fruity aroma as reported in literatures above.

The synthesis of acetic acid esters by *S. cerevisiae* yeasts during the fermentation of wine involves the activity of at least three acetyltransferases, namely; alcohol acetyltransferase, ethanol acetyltransferase and isoamyl alcohol acetyltransferase (Fukuda et al., 1998; Lilly et al, 2000). As seen in **Figure 4-12** and **4-13**, the most acetic acid ester producer was strain VIN13, excluding acetic acid ethyl ester, followed by strains EC1118 and AWRI R2. It is likely to have a correlation between high formations of acetic acid esters and fast fermentation kinetics and time (**Figure 4-8**). In accordance with previous studies, the higher levels of 2-phenyl ethanol seemed to determine the greater production of acetic acid 2-phenyl ethyl ester (Nykänen, 1986; Antonelli et al., 1999; Herjavec et al., 2003; Vilanova et al., 2007). These wines might impart intensive banana aroma as the levels of acetic acid 3-methylbutyl ester were above the aroma threshold at 40 $\mu g\ L^{-1}$ (see reported literatures in **5.1.1**). The concentrations of the other acetic acid esters were within the normal values detected in wine. The highest formations of MCFAs and their ethyl esters by strains Alchemy I and II, 4F9, LVCB and LittoLevure (**Table 4-8**) seemed to be responsible for their sluggish or stuck fermentation (**Figure 4-8**). In fact, it was found that MCFAs and their ethyl esters including ethanol might inhibit the growth of yeast (Ravaglia & Delfini, 1993; Ribéreau-Gayon et al., 2006 b). In addition, the high concentrations of MCFAs might be the cause of a greater formation of their corresponding ethyl esters, which is in accordance with earlier studies (Antonelli et al., 1999; Hernández-Orte et al., 2006 a; Vilanova et al., 2007). Saerens et al. (2006) have suggested that the regulatory mechanisms might be active at the level of MCFA biosynthesis rather than esterification, but it is also strongly depending on the yeast strains. Differences in expression of the ethyl ester synthetic genes *EHT1* and *EEB1* or regulation of the balance between their ester synthetic and esterase activities could be involved. Nevertheless, high levels of MCFA ethyl esters detected in most wines may be responsible for pleasant fruity and floral aromas (see reported literatures in **5.1.1**).

Some differences existed between the yeast strains in terms of linalool (rose, lavender and camphorous aromas) liberation. It is likely that strains X5, VIN13, VL3 and Alchemy I were the greatest linalool releasing strains, while most yeast strains had a similar ability to release α-terpineol, *trans*- and *cis*-linalool oxide (**Figure 4-14**). Interestingly, the concentration of linalool in most wines might contribute to typical varietal aroma at the perception threshold of 15-50 $\mu g\ L^{-1}$ as reported in previous literatures in **5.1.1**. In agreement with several authors, some strains of *S. cerevisiae* posess β-glycosidase activity that can release monoterpenes from glycoconjugate forms during alcoholic fermentation (Hock et al., 1984; Günata et al., 1985; Delcroix et al., 1994; Hernández et al., 2003; Carrau et al., 2005; Swiegers & Pretorius, 2005; Ugliano et al., 2006; Loscos et al., 2007).

5.2 Effects of yeast strains and/or nutrient supplements on wine fermentation and quality of Sauvignon blanc grape wines

In the following chapters, the obtained results of two fermentation trials of Sauvignon blanc grape juice are discussed. The improvement of fermentation characteristics, metabolic products and volatile compounds of Sauvignon blanc wine fermentation by optimal choices of commercial *Saccharomyces* yeast strains and nutrient supplementation; nutrient source and concentration, are reported in section **5.2.1** to **5.2.2**.

5.2.1 Effect of nine commercial *Saccharomyces* yeast strains and two nutrient sources on fermentative characteristics, metabolic compounds and wine aroma of Sauvignon blanc grape wines

The fermentation kinetics differed among the nine commercial yeast strains without any response to the two nutrient supplementations (**Figure 4-15**). Strains AWRI R2, X5, VIN13, VL3, EC1118 and LVCB had fast fermentation kinetics and durations, whereas strains Sauvignon and VIN7 had inverse behaviours with less response to the nutrient source. Nonetheless, all fermentations completely finished with similar amounts of low residual sugar (below 1 g L^{-1}) and glycerol (**Figure 4-16**), which were within the range of levels normally reported in wines (see previous literatures in **5.1.1**). It has been established that strain VIN7 usually has slow-acting fermentation kinetics, which might be related to its hybrid nature (Erasmus et al., 2004; King et al., 2008). Nevertheless, supplementation with Fermaid E slightly enhanced faster fermentation kinetics than OptiWhite. It might be due to the fact that Fermaid E contains certain nutrient sources, sufficient vitamins and growth factors for cell growth and metabolism as discussed before in **5.1.1**.

Some differences existed between the treatment combinations in terms of production and/or utilization of organic acids (**Table 4-9**). The addition of Fermaid E seemed to reduce acetic acid formation, except for strain VIN7. This might be due to the fact that this nutrient source provided high nutrient availability, and consequently increased NADH production, which in turn reduced the need of the yeast cell to produce acetic acid to control redox balance as suggested by Vilanova et al. (2007). Although strain VIN7 produced the highest amounts of acetic acid, its final wine had no detectable spoilage of vinegar-like odour (Corison et al., 1979; Ribéreau-Gayon et al., 2006 b). In addition, most wines did not undergo malolactic fermentation as well as low concentrations of the other acids had less impact on the quality of the final wines as discussed in **5.1.1**.

It is worth noting that the nine yeast strains showed similar formations of acetaldehyde in both nitrogen sources at the levels below the sensory threshold value as previously reported in literatures in **5.1.1**. The high formation of α-ketoglutarate by strains Sauvignon and X5 as well as pyruvate by strain VIN7 significantly diminished by the addition of Fermaid E (**Figure 4-17**). As discussed in **5.1.1**, it might be due to the fact that Fermaid E contains thiamine, which is reported to effectively reduce the keto acid formation by decarboxylation. On the contrary, this nutrient addition tended to result in high levels of CS_2 and dimethyl disulphide in the detected wines (**Figure 4-18**). Nonetheless, the concentrations of these compounds detected in all wine samples had no influence on the quality and undesirable sulphur aroma (see reported literatures in **Table 2.5** and **5.1.1**). In addition, these findings indicated that strains EC1118, 4F9 and AWRI R2 did not produce detectable CS_2 and DMDS.

It is worth noting that the nine yeast strains showed similar formations of all higher alcohols, acetic acid esters, ethyl ester of butanoic acid and lactic acid, all MCFAs and hexanoic acid ethyl ester in either the Fermaid E or OptiWhite treatment (**Table 4-10**, **4-11**, **4-12** and **Figure 4-19**, **4-20**). Besides, the most important aromatic higher alcohol is 2-phenyl ethanol, which has a floral fragrance and rose-like aroma (see previous literatures in **5.1.1**). Its concentrations in most wines were above the aroma threshold at 10 mg L^{-1}, while low formation of the other higher alcohols may impart desirable fruity aroma and aromatic complexity. Although the esters were directly derived from the corresponding higher alcohol through condensation with acetyl-CoA (Bell & Henschke, 2005; Vilanova et al., 2007), the production of acetic acid esters in this trial differed from the higher alcohols in response to nitrogen supplementation (**Table 4-10** and **Figure 4-19**).

The result revealed that acetic acid ester production was slightly higher with the addition of Fermaid E rather than OptiWhite for most yeast strains. This might be due to the fact that Fermaid E provides higher nutrient availability that consequently stimulates the formation of the alcohol acyl transferase enzymes promoting acetic acid ester biosynthesis as discussed before in **5.1.1**. A high concentration of most acetic acid esters in both nutrient sources was produced by strain Sauvignon only. This might be related to the distinctive capacity of the yeast to assimilate nitrogen and nitrogen sources. The yeasts with greater demand for nitrogen produce higher concentrations of esters during the fermentation (Perez-Coello et al., 1999; Torrea et al., 2003; Hernandez-Orte et al., 2005). It is likely that slow fermentation kinetics and time for strain Sauvignon (**Figure 4-15**) showed its higher nutrient demand and resulted in higher production of acetic acid esters in final wines. This finding demonstrated that only concentrations of acetic acid 3-methylbutyl ester (banana aroma) were beyond the aroma threshold (see reported literatures in **5.1.1**).

It should be emphasized that the addition of Fermaid E promoted the formation of ethyl ester of propionic acid, octanoic acid and decanoic acid (**Table 4-11** and **Figure 4-20**). As discussed in **5.1.1**, the increase in volatile ester formation is directly related to nitrogen availability and DAP addition at high level. On the other hand, the presence of OptiWhite seemed to increase the formation of isobutanoic acid ethyl ester and succinic acid diethyl ester, particularly by strains EC1118 and AWRI R2, respectively. Some observations reported similar results that concentrations of branched chain esters decrease with an increased DAP addition (Ugliano et al., 2007). Rosi et al. (2008) also observed that addition of organic nitrogen (yeast extract) stimulated yeast cells to produce high amounts of ethyl ester. Interestingly, high levels of these esters detected in the wine samples may contribute to the wine flavour of pleasant fruity and floral aroma (see reported literatures in **5.1.1**).

Most yeast strains had a similar capability to liberate small amounts of α-terpineol in both nutrient sources (**Table 4-11**) and its concentrations were quite below the aroma threshold at 400 µg L^{-1} as previously reported in literatures (**Table 2-4**). In addition, *trans*-linalool oxide, *cis*-linalool oxide and linalool, were detected only in trace quantities (data not shown). Großmann et al. (1990) and Swiegers et al. (2005 a) have suggested that under normal wine making conditions, endogenous β-glycosidases from the yeast have a limited effect on the development of the juice varietal aroma as discussed before in **5.1.1**.

5.2.2 Impact of five commercial yeast strains and four nutrient sources on fermentative characteristics, metabolic compounds and wine aromas of Sauvignon blanc grape wines

Fermentations of fresh juice with five yeast strains (EC1118, X5, VIN7, Alchemy I and LittoLevure) exhibited some significant differences of fermentation characteristics depending on the nutrient sources (Superstart, Fermaid O, Fermaid E and VUltra).

Strains EC1118 and Alchemy I were rather not influenced by the nitrogen source with fast fermentation kinetics and times (**Figure 4-21**). They completely finished fermentations with low residual sugar below 3 g L^{-1} (**Table 4-13**). In contrast, strains X5, LittoLevure and VIN7 were more influenced by the nitrogen source, which resulted in sluggish fermentations in the Fermaid E and Fermaid O variants. They afterwards finished fermentations with slightly high residual sugar (3.8-7.4 g L^{-1}). Nevertheless, Superstart seemed to be the most effective nutrient as it accelerated fermentation kinetics and time for all yeast strains. It has been suggested that PUFAs (polyunsaturated fatty acids) from inactive dry yeasts (IDY) can act as protective agents reducing the osmotic shock of active dry yeasts (ADY) because of the high sugar content of juices and helping yeasts to adapt their metabolism to the new conditions

(Caridi et al., 1999; Caridi, 2002). Recently, Soubeyrand et al. (2005) showed that IDY could release fragments of yeast cell walls that can form micelle-like particles, which consequently repair damaged cellular membranes of the yeast and are therefore able to increase the fermentation rate. On the other hand, the addition of Fermaid O and Fermaid E gave an inverse response, they retarded fermentation kinetics for strains X5, VIN7 and LittoLevure. It seemed to reflect their specific behaviour towards nitrogen source and consumption. In addition, the use of ammonium salts to increase the nitrogen content of grape must induced a repression of amino acid consumption by the yeasts and could reduce the fermentation efficiency, but this was strain-dependent (Bely et al., 1990; ter Shure et al, 2000; Beltran et al., 2005). The metabolism of nitrogen through the nitrogen transporter permeases, encoding *GAP1* (the transport of amino acids) and *MEP2* (the transport of ammonium), was repressed in a nitrogen-rich medium by the nitrogen catabolite repression (NCR) mechanism.

The glycerol and citric acid were not influenced by both yeast strain and nutrient source under the conditions of this trial (**Table 4-13** and **4-14**). There were some differences between the variants in the production and/or utilization of the other organic acids. Nevertheless, their levels in the wines were within the usual values in wine (see reported literatures in **5.1.1**). All treatments had no malolactic fermentation because of low lactic acid levels. It was exceptional for strain VIN7 that produced excess amounts of acetic acid above the detectable spoilage level of vinegar-like character, particularly in the Superstart variant.
Addition of Superstart resulted in a high formation of α-ketoglutarate and pyruvate but a low level of acetaldehyde for most yeast strains (**Figure 4-22**). Pozo-Bayón et al. (2009 a, 2009 b) have demonstrated that most IDY products provide amino acids, particularly glutamic acid. When glutamic acid is supplied as the sole major source of nitrogen, the α-amino group is mobilised in transamination reactions resulting in the high production of keto acids (Albers et al., 1996). This is shown in **Figure 2-2**. The addition of Fermaid O, VUltra and Fermaid E nonetheless diminished the formation of both keto acids. It might be due to the fact that Fermaid E and VUltra contain thiamine, which effectively decrease keto acids as discussed before in **5.1.1**. There was a positive correlation between high levels of SO_2 and a high formation of acetaldehyde (**Figure 4-22** and **4-23**). It was demonstrated that additional acetaldehyde is liberated in the presence of excessive quantities of sulphur dioxide in fermentation juices (Rankine & Pocock, 1969; Bell & Henschke, 2005; Ribéreau-Gayon et al., 2006 a). Nevertheless, the concentrations of these compounds detected in all treatments had no impact on the quality and stability of the final wines (see reported literatures in **5.1.1**).
The addition of Fermaid E and VUltra into the juice resulted in the highest formation of SO_2 and CS_2 by strain EC1118, while H_2S and dimethyl sulphide formations were similar among treatments (**Figure 4-23** and **4-24**). Sulphite formation by *Saccharomyces* yeasts is

influenced by the nutrient composition of the grape juice and by the content of sulphate (Rauhut, 2009). Strain X5 produced exceptionally high amounts of SO_2 in most nutrient conditions. It might possibly reflect the specific SO_2-forming yeast of this strain. A high formation of sulphite can be caused by defects in sulphate uptake and reduction (**Figure 2-8**). It could be demonstrated that in high-sulphite-producing strains sulphate-permease is not suppressed by methionine (Henschke & Jiranek, 1991; Rauhut, 1993; Jiranek et al. 1995 a, 1995 b; Pretorius, 2000; Rauhut, 2009). Nonetheless, levels of these sulphur compounds were not the cause of undesirable aromas in the final wines (see literatures in **Table 2-5**).

The production of higher alcohols was similar for most strains, which produced high amounts in the Superstart treatment but decreased with the addition of Fermaid E and VUltra (**Figure 4-25**). It is likely that Superstart provided less nitrogen availability and vitamin than the other nutrient sources. Surplus of α-keto acids, mainly synthesised from sugars, is consequently excreted as higher alcohols because of the shortage of α-amino nitrogen required for the transamination step of amino acid biosynthesis (Gutierrez, 1993; Oshita et al., 1995; Wang et al., 2003; Betran et al., 2005; Vilanova et al., 2007; Carrau et al., 2008). It was exceptional for the high production of individual higher alcohols by strains EC1118, Alchemy I and LittoLevure in most nutrient sources. It might nonetheless reflect a less efficient usage of nitrogen, which resulted in an increase of carbon flux related to branched-chain amino acid metabolism and carbon metabolic wastes including higher alcohols (Ribereau-Gayon et al., 2006 a; Carrau et al., 2008). The 2-phenyl ethanol, which is the most important higher alcohol, was obvious in the EC1118 wines and the VIN7 wines with the Superstart treatment at the levels above its aroma threshold. While levels of the other higher alcohols were below an undesirable level at 300 mg L^{-1} (see reported literatures in **5.1.1**).

Strain Alchemy I was the predominant producer of most acetic acid esters with less response to the nutrient source, except for acetic acid 2-phenyl ethyl ester (**Figure 4-26**). Strain VIN7 followed similar pattern in respect to acetic acid ethyl ester. The formation of acetic acid esters tended to increase with Superstart treatment, but decreased with Fermaid E and VUltra addition. It was suggested that polyunsaturated fatty acids from IDY like Superstart can act as protective agents reducing the stress of the yeasts and helping yeasts to adapt their metabolism to the new conditions (Caridi et al., 1999; Caridi, 2002; Soubeyrand et al., 2005). Thus, it might enhance yeast activity and metabolism to produce high levels of secondary metabolites as acetic acid esters. It is likely that the higher acetic acid ethyl ester production can be determined by the greater amount of acetic acid formed. Nevertheless, excessive levels of this ester produced by strains EC1118, Alchemy I and VIN7 were above the threshold of a positive impact (less than 160 mg L^{-1}), thus it might lead to an unpleasant volatile acidity, pungent and nail polish aromas (see reported literatures in **5.1.1**).

The EC1118 strain tended to be the most producer of ethyl ester of propionic acid and butanoic acid, MCFAs and their ethyl esters with less response to the nutrient source (**Table 4-15**, **4-16** and **Figure 4-27**). Strain Alchemy I followed similar pattern in respect to propionic acid ethyl ester production, while strain X5 produced high amounts of MCFAs and their ethyl esters. It seemed that there is a correlation between the high formation of these MCFAs and ethyl esters and the fast fermentation kinetics of the two strains above (**Figure 4-21**). The higher metabolic activities might also regulate the metabolic pathways of ester formations as suggested by some authors (Henschke & Jiranek, 1993; Albers et al., 1996; Wang et al., 2003; Hernández-Orte et al., 2005, 2006 a, 2006 b; Miller et al., 2007). The effect of Fermaid E on a high formation of ethyl ester of isobutanoic acid and lactic acid by strains Alchemy I and LittoLevure was consistent according to some studies (Hernández-Orte et al., 2005; Miller et al., 2007; Rosi et al., 2008) and previously discussed before in **5.2.1**. The addition of Fermaid O also promoted strains Alchemy I and LittoLevure to develop high amounts of these esters. Raynal et al. (2009) have reported that Fermaid O released 100 % of amino acids to the fermentation juice. As amino acids are precursors of some esters, they might increase the level of ethyl esters (Nykänen, 1986; Hernández-Orte et al., 2005, 2006 b; Miller et al., 2007).

The results also demonstrated that the rate of conversion of MCFAs into their corresponding ethyl esters is strongly dependent on the yeast strain. Differences in expression of the ethyl ester synthetic genes *EHT1* and *EEB1* or regulation of the balance between their ester synthetic and esterase activities could be involved (Hernández-Orte et al., 2005, 2006 a; Saerens et al., 2006; Vilanova et al., 2007). Interestingly, concentrations of ethyl esters of butanoic acid and MCFAs detected in the wines were above their aroma thresholds, which contribute to floral and fruity aromas as previously reported in literatures in **5.1.1**.

In general, the final wines of this Sauvignon blanc trial showed small amounts of monoterpene precursors, as most monoterpenes, e.g. α-terpineol, *trans*-linalool oxide, *cis*-linalool oxide and linalool were not detected.

5.3 Effects of yeast strains and nutrient supplements on wine fermentation and quality of yellow passion fruit wines

The following chapters are the outcome of four fermentation trials of yellow passion fruit juice. The effects of commercial *Saccharomyces* yeast strains and/or nutrient sources at different concentrations on the fermentation characteristics, metabolite by-products and volatile compounds of each fermentation trial are discussed in chapter **5.3.1** to **5.3.4**.

5.3.1 Effect of fourteen commercial *Saccharomyces* yeast strains on fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

Fourteen strains of commercial *Saccharomyces* yeasts (EC1118, AWRI R2, LittoLevure, QA23, Freddo, Sauvignon, VL3, X5, X16, VIN13, 4F9, LVCB, Alchemy I and II) were examined with regard to their impacts on the fermentation characteristics and formation of metabolic compounds of obtained yellow passion fruit (YPF) wines. The results showed that the QA23 strain had a similar fast fermentation kinetic and time as the LVCB and VIN13 strains (**Figure 4-28**) and was the lowest pyruvate, α-ketoglutarate and glycerol producer as well (**Figure 4-29** and **4-30**). In contrast, strains VL3, X16, LittoLevure, Sauvignon and AWRI R2 had slow and sluggish fermentations. However, all YPF fermentations completely finished with low residual sugar at less than 2 g L^{-1} (**Figure 4-29**). Commercial yeast strains have very different nitrogen demands, and consequently affect the fermentation kinetics and characteristics as reported in some studies (Julien et al., 2000; Pretorius, 2000; Blateyron & Sablayrolles, 2001; Colombié et al., 2005; Beltran et al., 2005) and previously discussed in **5.1.2**. In addition, a sluggish fermentation can be due to nitrogen deficiency, thiamine depletion of the must, CO_2 and ethanol toxicity (see reported literatures in **5.2.1**). A slow and sluggish fermentation seemed to determine the greater formation of glycerol in YPF wines, as found in the X16, AWRI R2 and LittoLevure YPF wines (**Figure 4-29**). Several authors have suggested that the two most important functions of glycerol synthesis in yeast are related to redox balancing and the hyperosmotic stress response that its formation is increased during osmotic stress (Scanes et al., 1998; Swiegers et al., 2005 a; Ribéreau-Gayon et al., 2006 a; Vilanova et al., 2007). Glycerol concentrations in these YPF wines were nonetheless similar to the normal grape wine value (see previous literatures in **5.2.1**).

Some differences existed between the treatment combinations in terms of production and/or utilization of organic acids (**Table 4-17**). Although the Alchemy I, II and AWRI R2 strains produced high amounts of acetic acid, their finished YPF wines had no detectable spoilage of vinegar-like odour (see previous literatures in **5.2.1**). In addition, most YPF wines did not undergo malolactic fermentation because of no detectable lactic acid. It is worth noting that the occurrence of large amounts of citric acid in YPF wines originates from the natural YPF juice itself (see reported data in **3.9.1**), which was previously reported in the fruit juice fermentation by Jitjaroen (2007) and Srisamatthakarn et al. (2010).

The overproduction of α-ketoglutarate and pyruvate by strains Sauvignon and X16 might be related to their slow fermentation kinetics and higher nutrient demand (**Figure 4-28** and **4-30**). Some authors have reviewed that when nitrogen is adequate keto acids typically accumulate in wine to less than 50-100 mg L^{-1}, when nitrogen is limited, more than 100 mg L^{-1} can be

produced by yeasts (Rankine, 1968 b; Radler, 1993; Hernández-Orte et al., 2005). On the contrary, strains 4F9 and Freddo that had fast fermentation kinetics also developed high amounts of these keto acids. The reason for this could be an extreme deficiency of vitamins in the YPF juice like thiamine and pantothenic acid (Delfini et al., 1980; Bataillon et al., 1996; Ribéreau-Gayon et al., 2006 a). In addition, excessive levels of these keto acids might affect the stability and quality of YPF wines (see reported literatures in **5.1.1**).

There was an inverse correlation between a high acetic acid and a low acetaldehyde formation by the EC1118, 4F9 and QA23 strains (**Table 4-17** and **Figure 4-30**). It has been reported that acetaldehyde can be converted to ethanol and acetic acid through alcohol dehydrogenase enzymes (Pronk et al., 1996; Marks et al., 2003). In addition, the ability of yeast strains to produce acetaldehyde is depending on the activity of the enzyme, alcohol dehydrogenase, involved in its synthesis (Pérez-Coello et al., 1999; Remize et al., 1999; Cheraiti et al., 2005; Regodón Mateos et al., 2006). Nonetheless, its levels in all YPF wines were within the reported wine values and it did neither contribute to the green, bruised apple and nutty flavours nor led to a wine oxidation (see previous literatures in **5.1.1**).

A low level of carbon disulphide in the QA23, X5, 4F9, EC1118 strains might be related to their fast fermentation kinetics (**Figure 4-28**), while the LittoLevure strain had an inverse behaviour and high amounts (**Figure 4-31**). It is likely that a slow and sluggish fermentation of strain LittoLevure may lead to a high formation of MeSAc. As discussed before in **5.1.2**, yeasts with a low vitality such as in sluggish fermentations can tend to autolyse faster, and consequently lead to the formation of several sulphur compounds (Berry & Watson, 1987; Henschke & Jiranek, 1993). On the other hand, strain VIN13 also developed high amounts of MeSAc, although it had a fast growth kinetic, thus some specific genes might be involved. Interestingly, MeSAc was not detected in the YPF wines fermented with strains EC1118, Sauvignon, VL3, X5, Alchemy I, 4F9, QA23 and Freddo. The concentration of these compounds in the YPF wines had no impact on the sulphurous off-flavour (see reported literatures in **Table 2-5**).

As discussed in the previous trial (**5.1.2**), the use of different yeast strains during fermentation contributed considerably to variations in individual volatile compound formations. It seemed that high productions of higher alcohols by strains LittoLevure, AWRI R2, X16 and Sauvignon were related to the slow fermentation kinetics (**Figure 4-28** and **4-32**). On the other hand, it can be suggested that the high formation of 2-phenyl ethanol and 2-methyl butanol by strains QA23 and VIN13, which had fast fermentation kinetics, might reflect a less efficient usage of nitrogen. Thus, it resulted in an increase of carbon flux related to branched-chain amino acid metabolism and carbon metabolic wastes including higher alcohols

(Vilanova et al., 2007; Carrau et al., 2008). Nevertheless, concentrations of these higher alcohols in YPF wines might be considered as desirable flavours as their levels were below 300 mg L^{-1} (see reported literatures in **5.1.1**). In addition, the LittoLevure, QA23 and VIN13 YPF wines may impart floral and rose aromas because of high levels of 2-phenyl ethanol.

The LittoLevure strain followed by strains QA23, VIN13, X16 and LVCB was the most predominant producer of acetic acid esters in the YPF wines (**Figure 4-33**). The acetic acid ethyl ester is present as relative peak area due to the low detected amounts in the YPF wines. It is likely that the higher amounts of higher alcohols were responsible for the greater formation of corresponding acetic acid esters as discussed before in the Scheurebe grape wine trial (**5.1.2**). The concentrations of only acetic acid 3-methylbutyl ester in the YPF wines were quite above the aroma threshold (banana and fruity aromas) as reported in literatures in **5.1.1**.

There was a relationship between the high formation of ethyl ester of lactic and isobutanoic acid by strains X16, Sauvignon and AWRI R2 (**Figure 4-34**) and their slow fermentation kinetics (**Figure 4-28**) as well as their distinctive capacities to assimilate nitrogen. It has been suggested that the yeasts with greater demand for nitrogen/nutrients produced higher levels of esters during fermentation (Perez-Coello et al., 1999; Torrea et al., 2003). On the other hand, strains QA23, Freddo and LVCB developed high amounts of butanoic acid ethyl ester, MCFAs and their esters (**Table 4-18** and **Figure 4-34**, **4-36**), while strains X5 and EC1118 produced high levels of propionic acid ethyl ester, but they had fast fermentation kinetics. The higher metabolic activities of yeasts may also regulate the metabolic pathways of ethyl ester formations as suggested by some studies (Albers et al., 1996; Hernández-Orte et al., 2005, 2006 a, 2006 b; Miller et al., 2007). In addition, the level of MCFA biosynthesis seemed to be responsible for the formation of related ethyl esters in the YPF wines as discussed in previous trials of grape wines (**5.1** and **5.2**). The concentrations of most ethyl esters detected in YPF wines, except for propionic acid ethyl ester, may impart the fruity and floral aromas (see previous literatures in **5.1.1**) but levels of MCFAs (soapy and rancid odours) were far away from their thresholds.

This finding showed that most yeast strains had a similar ability to liberate α-terpineol (**Figure 4-35**). The linalool was most evident in the QA23, Alchemy I and II and 4F9 YPF wines. It seemed that the higher metabolic activities of these yeast strains, which had the faster fermentation kinetics, stimulated the greater release of linalool. Nonetheless, concentrations of these monoterpenes were quite below their perception thresholds as reported in the literatures (**Table 2-4**).

5.3.2 Effect of three commercial *Saccharomyces* yeast strains and five different commercial nutrient sources on growth kinetics, fermentative characteristics, metabolic compounds and aromas of yellow passion fruit wines

The YPF juice fermentations with three yeast strains (EC1118, Sauvignon and X5) showed some significant differences on the fermentation kinetics, metabolic and volatile compounds depending on the nutrient sources (DAP, VCombi, VUltra, Fermaid E and OptiWhite).
Addition of any source of nutrient, except OptiWhite, into YPF juices seemed to be effective to enhance the fermentation kinetics of most strains, except for strain Sauvignon (**Figure 4-37**). Nevertheless, all YPF fermentations completely finished with low residual sugar (less than 2 g L^{-1}) and similar amounts of glycerol (**Table 4-19**). The effect of OptiWhite, which is an inactive dry yeast, might be due to the fact that it provides less nitrogen availability to the YPF juice. On the contrary, when a nutrient containing DAP was added to the YPF juice, resulting in an increase of ammonium nitrogen, *Saccharomyces* yeasts were possibly able to transport ammonium ions across the plasma membrane more easily than another nitrogen source (Miller et al., 2007; Boulton et al., 2009). Furthermore, addition of nutrient mixtures like VCombi, VUltra and Fermaid E accelerated fermentation kinetics faster than DAP alone as discussed before in **5.1.1** and **5.2.1**. It can be suggested that under the condition in this trial, the impact of one nutrient (e.g. assimilable nitrogen) on the fermentation kinetics during alcoholic fermentation is also dependent upon the other nutrients.

It has been demonstrated that malic acid is catalyzed by some strains of *S. cerevisiae* via sugar metabolism (Antonelli et al., 1999; Reynolds et al., 2001; Yéramian et al., 2007). As shown in **Table 4-20**, in the VCombi and DAP treatments strain Sauvignon catalyzed 39.5 to 64.7 % malic acid. Strain X5 also produced 39.5 % malic acid in the Fermaid E variant. The addition of these nutrients probably accelerated the fermentation kinetics and consequently regulated the other pathways including organic acid metabolism (Radler, 1993; Bell & Henschke, 2005; Ribéreau-Gayon et al., 2006 a; Ugliano et al., 2007). Acetic acid formation plays an important physiological role in the intracellular redox balance by regenerating reduced equivalents of NADH (Bely et al., 2003; Ribéreau-Gayon et al., 2006 a). Thus, an increase of acetic acid production by strain Sauvignon in the Fermaid E and OptiWhite variants, which had sluggish fermentations, might control the redox balance of this strain. Nonetheless, concentrations of these acids in the YPF wines were similar to the reported ranges in a normal grape wine (see literatures above) and these YPF wines did neither undergo malolactic fermentation nor had a vinegar-like odour. As previously discussed in **5.3.1**, high amounts of citric acid in YPF wines originate from the natural YPF juice itself (see reported data in **3.9.2**).

As previously discussed in **5.1.1** and **5.2.2**, the addition of a nutrient source containing thiamine; VCombi, VUltra and Fermaid E, significantly diminished the formation of α-ketoglutarate and pyruvate in the YPF wines (**Figure 4-38**). Jitjaroen (2007) also demonstrated that the fermentation of fruit wines, santol and ma-mao, with DAP plus thiamine and Fermaid E reduced the production of keto acids. The excessive formation of α-ketoglutarate by strains Sauvignon and EC1118 in the DAP treatment was quite above the normal wine value, thus it might affect the stability and quality of these YPF wines (see reported literatures in **5.1.1**). There was probably a correlation between the high formation of acetaldehyde and the fast fermentation kinetics of strain EC1118 with the addition of VCombi, VUltra and Fermaid E (**Figure 4-37**). Recently, Jitjaroen (2007) reported a similar result that the addition of Fermaid E promoted greater yeast growth rates and acetaldehyde production in some fruit wines than DAP alone. Nevertheless, levels of acetaldehyde and pyruvate in YPF wines were similar to the reported ranges in grape wine.

Ammonium salts like DAP are widely used to compensate nitrogen deficiencies in the juice and to practically control H_2S formation (Hansen et al, 1994; Rauhut, 2009). Nevertheless, its supplementation is not always effecting H_2S production because of other factors, e.g. methionine and other nitrogen that regulate amino acid transport into the yeast cell and sulphur metabolism, especially the SRS pathway, which generates H_2S (Spiropoulos & Bisson, 2000; Spiropoulos et al., 2000) and deficiencies in vitamins and micronutrients (Wainwright, 1971; Wang et al., 2003). This is largely due to the fact that the regulation of sulphur and nitrogen metabolism in yeast is complex (Marks et al., 2003; Bell & Henschke, 2005). Thus, the high production of H_2S by strain EC1118 in the presence of DAP might be partially explained (**Figure 4-39**). Overall, H_2S and carbon disulphide formation decreased with the addition of a nutrient mixture containing thiamine as well as OptiWhite. Their levels in the YPF wines had nonetheless no undesirable sulphur off-flavour (**Table 2-5**). In contrast, the addition of VCombi and VUltra resulted in a high formation of methanethiol (MeSH) by strain EC1118. It is derived from methionine, cysteine, S-methylmethionine and sulphate (De Mora et al., 1986; Rauhut, 1993). The addition of these nutrient sources might therefore provide certain substrates for this strain to liberate high amounts of MeSH under this trial condition. However, ongoing research is necessary to prove the effect of nutrient sources on the sulphur compound formation in the YPF wines. The odour threshold of MeSH is extremely low at 0.02-0.3 μg L^{-1}, thus detected levels in these YPF wines may contribute to an undesirable aroma of rotten egg or cabbage.

It is well documented that an inverse correlation exists between DAP addition and the production of higher alcohols, as pathways leading to the alcohol synthesis are down-regulated by the addition of DAP (Rapp & Versini, 1991, 1996; Marks et al., 2003; Beltran et

al., 2005; Hernández-Orte et al., 2006 a). This result also showed that the addition of DAP significantly decreased the concentration of 2-methyl butanol and 2-phenyl ethanol for most yeast strains, but these alcohols increased with other nutrient mixtures (**Figure 4-40**). On the other hand, the DAP addition promoted high formations of 2-methyl propanol and 3-methyl butanol, particularly by strains Sauvignon and EC1118. Hernández-Orte et al. (2005) have suggested that DAP increases the capacity of the yeast to transform the synthesized α-keto acids, avoiding their accumulation and later expulsion to the medium after their reduction to higher alcohols. In addition, it may also reflect a less efficient usage of nitrogen for these strains (see similar discussion in **5.2.2**). For instance, strain Sauvignon seemed to be the predominant producer of higher alcohols, but their concentrations were within the desirable value in grape (see reported literatures in **5.1.1**). In addition, high levels of 2-phenyl ethanol produced by this strain may elicit floral and rose aroma in YPF wines.

Strain Sauvignon developed prominent aroma compounds in terms of acetic acid esters (excluding acetic acid hexyl ester), linalool and α-terpineol with the addition of nutrient sources containing thiamine (VCombi, VUltra and Fermaid E) (**Figure 4-41** and **4-42**). It has been reported that acetic acid ester can be derived directly from the corresponding higher alcohol through condensation with acetyl-CoA (Nykänen, 1986; Antonelli et al., 1999; Herjavec et al., 2003; Vilanova et al., 2007). This result also indicated that there was a positive relationship between higher concentrations of higher alcohols and the greater productions of corresponding acetic acid esters by strain Sauvignon with the same nutrient condition (**Figure 4-40** and **4-41**). Acetic acid hexyl ester is probably derived from a grape precursor, hexyl aldehyde that is modified by yeast metabolism (Boulton et al., 2009). Strains EC1118 and X5 with VCombi addition showed high liberation of this ester. High acetic acid 3-methylbutyl ester productions in the YPF wines, particularly in the Sauvignon treatments, may have an intensive banana aroma (see reported literatures in **5.1.1**). The slow fermentation time of strain Sauvignon seemed to correspond to high levels of α-terpineol and linalool. A longer fermentation time may lead to high release of monoterpenes under the condition in this trial. However, their concentrations were quite below the perception thresholds (**Table 2-4**).

Likewise, depending on the yeast strain utilized, the formation of ethyl ester compounds presented different responses when different nutrients were added to the medium as shown in previous studies (Torrea et al., 2005; Miller et al., 2007). Strain Sauvignon with DAP addition produced the highest amounts of ethyl esters of isobutanoic acid and lactic acid (**Table 4-21**). The addition of VCombi promoted strain EC1118 to develop the greatest concentrations of butanoic acid ethyl ester, medium-chain fatty acids and their ethyl esters as well as strain X5 with respect to fatty acids and their ethyl esters (**Table 4-22**). The propionic

acid ethyl ester was most obvious in the YPF wines produced by strain X5 with Fermaid E and OptiWhite additions. Similar to earlier studies (Hernández-Orte et al., 2005, 2006 a; Miller et al., 2007; Rosi et al., 2008) who suggested that a large pool of ammonium released from these nutrient sources was not used for cellular structures and growth of the yeast but was rather available to produce high levels of secondary metabolites as ethyl esters. The high formation of fatty acids and their ethyl esters by strains EC1118 and X5 (**Table 4-22**) seemed to be related to their fast fermentation kinetics in the same nutrient sources (**Figure 4-37**). As discussed in **5.2.2**, these nutrients enhanced the metabolic activities of the yeast, and might consequently regulate the metabolic pathways of ester formations as well. In addition, the higher concentration of fatty acids seemed to determine the greater production of corresponding fatty acid ethyl esters. Nevertheless, the rate of conversion is strongly dependent on the yeast strain as suggested by some research groups (Antonelli et al., 1999; Herjavec et al., 2003; Torija et al., 2003 a, 2003 b; Vilanova et al., 2007).
The YPF wines produced by most yeast strains with the addition of DAP and VCombi might have an odour reminiscent of floral and fruity aromas, as the concentration of ethyl esters in these samples were beyond the perception thresholds (see reported literatures in **5.1.1**).

5.3.3 Effect of five commercial *Saccharomyces* yeast strains and two different commercial nutrient sources at different concentrations on growth kinetics, fermentative characteristics, metabolic and aroma compounds of yellow passion fruit wines

The YPF puree had low pH and extremely high acidity, thus it was diluted by the addition of water to the optimal value for yeast growth and sensory preferences (Ribéreau-Gayon et al., 2006 a; Swiegers et al., 2005 a; www.foodsci.purdue.edu/research/labs/enology/Acid 2007slides.pdf, 2009) as shown in **Table 4-23**. Concentrations of α-amino nitrogen in the prepared YPF juice were consequently reduced to approximately 91.9 % and it had a low total amino acid and sugar content (**Table A-3**). The sugar content was then adjusted by adding sucrose (beet sugar) giving inverted sugar to 170.5 $\pm$ 12.1 g L^{-1}. In accordance with some authors, numerous higher alcohols and esters are produced during fermentation (Francis & Newton, 2005; Ribéreau-Gayon et al., 2006 b). These compounds were detected from traces to below 5 mg L^{-1} in the YPF puree and prepared juice and below the aroma threshold reported in the literatures above, except for levels of linalool that were above the perception threshold (15-50 μg L^{-1}). In addition**,** a decrease of the aroma compound concentrations in prepared YPF juice was also due to the addition of water to YPF puree.
The fermentations of prepared YPF juice were examined with regard to the effect of yeast strains (EC1118, VL3, X5, Alchemy I and LittoLevure), nutrient sources (DAP and VCombi)

and nutrient levels (0.25 and 0.5 g L^{-1}) on the fermentation characteristics, non-volatile and volatile compounds in the final YPF wine. The result indicated that strains EC1118 and Alchemy I had the fastest fermentation kinetics and durations, while strains LittoLevure and VL3 resulted in sluggish fermentations depending on the nutrient supplement (**Figure 4-43**). Nevertheless, these yeast strains fermented practically nearly all the initial sugar provided (**Table 4-23**) and resulted in a low residual sugar below 2 g L^{-1} (**Table 4-24**). The addition of VCombi at a high concentration (0.5 g L^{-1}) seemed to be the most effective condition to accelerate fermentation kinetics and time for the five yeast strains. The VCombi contains thiamine, which is also a growth factor hence it might help the yeast to adapt its metabolism to the new conditions as previously discussed in **5.3.2**. The addition of thiamine to the grape juice influences the growth rate, the velocity and the kinetic of fermentation (Delfini & Formica, 2001; Ribéreau-Gayon et al., 2006 a; Jitjaroen, 2007). On the contrary, strain LittoLevure showed slow fermentation kinetic in the VCombi at low level, it might be due to its less effective behaviour in relation to nitrogen usage as well as the nitrogen deficiency and thiamine depletion of the juice as suggested by some studies (see reported literatures in **5.2.2** and **5.3.2**).

High productions of glycerol by strains VL3 and X5 are probably related to their slow fermentation kinetics in the low DAP treatment (**Table 4-24** and **Figure 4-43**). Larsson et al. (1998) have suggested that when *Saccharomyces* yeast cells face nitrogen limitation, glycerol synthesis is increased in order that excessive cytosolic ATP can be consumed. In addition, glycerol may have a role in oxidative stress resistance, in regenerating cytosolic inorganic phosphate and in nitrogen metabolism (Scanes et al., 1998). Even though some variations in the glycerol production were observed among the treatments, glycerol levels in all YPF wines were within the optimal wine ranges (see reported literatures in **5.1.1**). Some differences existed among the treatment combinations in terms of citric and malic acid concentrations, except for acetic acid (**Table 4-25**). It might be due to a chemical reaction with other substances in the YPF wine. It has been reviewed that many yeasts are able to use certain carboxylic acids not only as sources of carbon for growth but also for controlling the intracellular pH and contribute therewith to the intracellular charge balance by enhancing K^+ ion uptake (Walker, 1998; Torija et al., 2003 b). The lactic acid was not detected in all YPF wines, thus they did not undergo malolactic fermentation. In addition, concentrations of organic acids were similar to the reported values in grape wines and YPF wines in **5.3.1** and **5.3.2**.

Excessive amounts of α-ketoglutarate and pyruvate in the YPF wines were produced by strains X5 and VL3 in the presence of DAP, especially at low levels, but significantly diminished with VCombi (DAP plus thiamine) addition (**Figure 4-44**). It has been suggested that when ammonium salts are added as sole major source, they will be converted to glutamate and mobilized in transamination reactions in yeasts resulting in the formation of other amino acids and keto acids, however depending on the yeast strain (Rankine, 1968 b; Albers et al., 1996). As previously discussed in **5.2.2** and **5.3.2**, thiamine effectively decreased keto acid productions. The result indicated that excessive levels of keto acids detected in the YPF wines produced by strains X5 and VL3 in the DAP treatment seemed to influence the stability and quality. The acetaldehyde formation in all the YPF wines showed no significant difference and was below the sensory threshold (see reported literatures in **5.1.1**).

Similar to the previous trial, although DAP was added to compensate nitrogen deficiencies in the YPF juices, high formations of H_2S by most strains were observed (**Figure 4-45**). Rauhut (2009) suggested that some yeast strains are constantly high or constantly low H_2S producers (see previous discussion in **5.3.2**). On the other hand, its production significantly decreased when the VCombi was supplemented into the juice at 0.5 g L^{-1}. Regarding CS_2, the slow and sluggish fermentations of strains LittoLevure and VL3 seemed to affect their high productions under similar nutrient conditions. On the contrary, high production of MeSH seemed to be related to fast fermentation kinetics of strains EC1118 and Alchemy I under the same nutrient conditions (**Figure 4-43** and **4-45**). It is likely that the accelerated fermentation kinetic and yeast activity regulated the liberation of MeSH. In addition, a high formation of H_2S by both strains in the same nutrient condition might lead to a high production of MeSH as well as a high production of MeSAc by the Alchemy I strain. It has been reported that a high H_2S formation in the early phase of fermentation leads to high amounts of MeSAc (Matsui & Amaha, 1981; Rauhut, 1996). Although concentrations of H_2S, CS_2 and MeSAc detected in the YPF wines were below the aroma threshold (**Table 2-5**), levels of MeSH produced by strains EC1118 and Alchemy I under certain nutrient conditions may give a reductive off-flavour (odour threshold 0.02-0.3 μg L^{-1}) in YPF wines.

The result in **Figure 4-46** indicated that hexanol amounts in YPF wines were similar among treatments. As previously discussed in **5.3.2**, the addition of DAP at high level resulted in the highest production of 2-phenyl ethanol, 2-methyl and 3-methyl butanol by strain LittoLevure, as well as 2-methyl propanol by strain VL3. As previously suggested in **5.3.2**, addition of DAP increased the capacity of some yeast strains to transform the synthesized α-keto acids, avoiding their accumulation, but reduced them to higher alcohols (Beltran et al., 2005; Hernández-Orte et al., 2005, 2006 a). In contrast, the addition of VCombi diminished keto

acids and reduced concentrations of higher alcohols, except for 2-phenyl ethanol, for all yeast strains. The higher alcohol production by wine yeasts appears to be the result of either the anabolic formation of amino acids *de novo* from a sugar substrate or the catabolic conversion of the branched-chain amino acids via corresponding keto acids from the Ehrlich pathway (Äyräpää, 1971; Rapp & Versini, 1991, 1996; Marks et al., 2003; Bell & Henschke, 2005; Ribéreau-Gayon et al., 2006 a). The high formation of 2-phenyl ethanol by strain LittoLevure with less response to the nutrient supplement might reflect a less efficient usage of nitrogen as previously discussed in **5.2.2** and **5.3.2**. The levels of higher alcohols in YPF wines were below 300 mg L^{-1}, which are usually considered desirable (see reported literatures in **5.1.1**).

Even though the YPF wines produced by strains VL3 and X5 in most nutrient supplements showed a tendency to develop high amounts of acetic acid ethyl ester, their concentrations were below the quantification limit as displayed in relative peak area (**Figure 4-47**). Strain LittoLevure, with the addition of either DAP or VCombi at high level, produced prominent acetic acid esters. The addition of high level of VCombi resulted in the greatest formation of acetic acid 3-methylbutyl ester by strains VL3 and acetic acid 2-methylbutyl ester for all yeast strains. As previously discussed in **5.2.2** and **5.3.2**, a high nutrient level promoted a great formation of acetic acid esters. It is likely that VCombi, which enhanced the metabolic activity of the yeast, also regulated the metabolic pathways of acetic ester production. In addition, the high formation of 2-phenyl ethanol by strain LittoLevure in YPF wines yielded a great amount of acetic acid 2-phenyl ethyl ester above the aroma threshold in wine (see previous literatures in **5.1.1**). These YPF wines might also impart an intensive banana aroma as the concentrations of acetic acid 3-methylbutyl ester were quite above the threshold.

As demonstrated in **Table 4-26**, strain X5 produced constantly high amounts of propionic acid ethyl ester with less response to the nutrient supplementation. It might be due to an efficient behaviour of each strain in relation to the distinctive capacity of nitrogen usage (Carrau et al., 2008). High formations of lactic acid ethyl ester by strains VL3 and X5 in the high DAP level, isobutanoic acid ethyl ester by strain VL3 in the DAP treatment and butanoic acid ethyl ester by strains EC1118 and X5 in a high level of VCombi seemed to be related to their fast fermentation kinetics. As previously discussed in **5.3.2**, the addition of ammonium into the YPF juice, particularly at high level, promoted high formations of ethyl esters. Lactic acid ethyl ester is known to be formed from lactic acid and ethanol (Mason & Dufour, 2000; Sumby et al., 2009), while lactic acid can be derived from pyruvate, directly reduced by yeast lacticodehydrogenases (Ribéreau-Gayon et al., 2006 a). Thus, the addition of VCombi, which reduced the formation of pyruvate (**Figure 4-44**), might be involved in the significant decrease of lactic acid ethyl ester production in YPF wines. High concentrations of these

ethyl esters detected in certain YPF wines may elicit pleasant fruity and floral aromas (see reported literatures in **5.1.1**). Likewise, the addition of VCombi at high level, which enhanced fast fermentation kinetics and metabolic activities of strains EC1118 and X5, resulted in a high production of MCFAs and ethyl esters (**Table 4-27**). In addition, high levels of MCFAs produced by the two strains seemed to be responsible for the greater production of related ethyl esters, which is similar to the previous discussion in **5.3.2**. Nevertheless, concentrations of only ethyl ester of hexanoic acid and octanoic acid were beyond the odour threshold.

Even though the yeast strains with nutritive supplementation had an influence on some volatile compounds, they released similar amounts of linalool and α-terpineol in the YPF wines with no response to the nutrient supplement (**Figure 4-48**). Their concentrations in the YPF wines were quite below the aroma threshold (**Table 2-4**). A limited effect of endogenous β-glycosidases on the development of the juice aroma due to its activity is optimum at pH 5 (Großmann et al., 1990; Swiegers et al., 2005 a) and the clarification of YPF juice might inhibit its activity.

5.3.4 Effect of three commercial *Saccharomyces* yeast strains and four different nutrient sources at two different concentrations on growth kinetics, fermentative characteristics, metabolic compounds and aroma of yellow passion fruit wines

As previously discussed in **5.3.3**, the YPF juice used for the fermentation was diluted by the addition of 10-fold water because its natural puree has high acidity (**Table 4-28**). The YPF juice then had a low α-amino nitrogen, total amino acid and sugar content (**Table 4-28** and **A-3**), and beet sugar was added into the juice resulting in 170.0 $\pm$ 2.8 g L^{-1} inverted sugar. The yeast nutrient was supplemented according to the experimental plan (**Table 3-12**) to compensate nutrient deficiencies in the YPF juice. The results also indicated that concentrations of volatile compounds in prepared YPF juice were detected in small amounts below the aroma threshold (see reported literatures in **5.1.1**).

Strain EC1118 had shorter fermentation duration with less response to the nutrient addition than strains X5 and VL3 (**Figure 4-49**). Strain VL3 with the addition of 0.4 g L^{-1} OptiWhite resulted in a slow and stuck fermentation with residual sugar higher than 10 g L^{-1} (**Table 4-29**). It is likely that strains VL3 and X5 were more sensitive to the nutrient source and the concentration than strain EC1118, and had a higher nitrogen demand. Nevertheless, the VCombi and VUltra at high level seemed to be the most effective to enhance fermentation kinetics for all yeast strains in the YPF juice fermentations, while OptiWhite was the least effective, particularly at low level. Similar to previous trials, the nutrient mixtures containing thiamine and growth factors gave better fermentation kinetics than the single nutrient source

(see reported literatures in **5.2.2** and **5.3.2**). On the other hand, some yeast strains resulted in sluggish or stuck fermentations with the addition of Fermaid E. It might be due to a specific nitrogen demand of each yeast strain, nitrogen deficiency, thiamine depletion and an excessive clarification of the YPF juice as previously discussed in **5.3.3**. Even though both yeast strain and nutrient supplement affected fermentation kinetics and residual sugar content in the YPF wines, they had no significant influence on the glycerol production (**Table 4-29**).

There were some differences among the treatments in terms of organic acid concentrations in the YPF wines, but they had no impact on the quality (**Table 4-30**). Nevertheless, concentrations of these acids in YPF wines were normally within the reported wine ranges (see previous literatures in **5.1.1**). It was exceptional that high citric acid amounts in the YPF wines were originally from the YPF juice itself as previously discussed in the YPF trials.

Similar to previous trials, the addition of OptiWhite at both levels, resulted in excessive productions of α-ketoglutarate and pyruvate, particularly by strain VL3 (**Figure 4-50**). Pozo-Bayón et al. (2009 a, 2009 b) have suggested that the IDY product like OptiWhite can release amino acids, particularly glutamic acid that is the donor of nitrogen in several biosynthetic pathways of amino acids. An excess of α-ketoglutarate can consequently be formed as a de-amination product (Albers et al., 1996; Rankine, 1968 b). On the other hand, the addition of other nutrient sources containing thiamine significantly reduced the formations of both keto acids as previously discussed in several trials. The excessive amounts of both keto acids in the YPF wines produced by strains VL3 and X5 with OptiWhite addition might affect the stability and quality (see previous literatures in **5.1.1**). Under this trial condition, all yeast strains produced similar amounts of acetaldehyde without any response to nutrient addition and had no influence on the quality of the YPF wines.

The result indicated that the YPF juice supplemented with OptiWhite, which is rich in glutathione, resulted in high production of H_2S (**Figure 4-51**). Tokuyama et al. (1973) suggested that when nitrogen is limited, H_2S can be produced by the degradation of glutathione and the release of cysteine. In addition, the impact of vitamins or degradation of sulphur reserves like glutathione is involved in H_2S formation (Eschenbruch, 1974; Henschke & Jiranek, 1993; Hallinan et al., 1999; Wang et al., 2003; Edwards & Bohlscheid, 2007). Nevertheless, production of H_2S significantly diminished when another nutrient source containing DAP was added into the YPF juice. It is likely to have a relationship between high concentrations of carbon disulphide and sluggish fermentations of strains X5 and VL3 with the addition of low Fermaid E level, but the mechanism involved is unclear. Strain EC1118 with a high level of VUltra and Fermaid E addition produced methanethiol in the YPF wines, which seemed to be related to the fast fermentation kinetics. The accelerated fermentation

activity of this strain might have regulated the liberation of MeSH. Although levels of H_2S and CS_2 were not high enough to cause a reductive sulphur off-flavour in the YPF wines, levels of MeSH in certain EC1118 samples were above its odour threshold (**Table 2-5**) and might impart a reductive odour of rotten eggs.

As shown in **Figure 4-52**, the effect of the nutrient source at different concentrations on higher alcohol production was dependent upon the inoculated yeast strain, except for hexanol. Strain VL3 with high OptiWhite addition developed the prominent concentrations of 2-methyl propanol and 3-methyl butanol. As previously discussed in **5.3.2**, higher formations of α-ketoglutarate by strain VL3 (**Figure 4-50**) seemed to be responsible for the greater amount of these alcohols in the low OptiWhite sample. Higher alcohols can be formed from intermediates in branched-chain amino acids via corresponding keto acids from the Ehrlich pathway (Bell & Henschke, 2005). On the other hand, the addition of Fermaid E at both levels promoted the greatest production of 2-phenyl ethanol by strain EC1118 in the YPF wines and 2-methyl butanol by strain VL3. The former compound was also most obvious in the high VCombi treatment of strain EC1118. Nevertheless, the effect of the nutrient source containing DAP on the high production of some higher alcohols is earlier discussed in **5.3.2** and **5.3.3**. Concentrations of hexanol were not affected from both yeast strain and nutrient supplement. Clarke & Bakker (2004) and Bell & Henschke (2005) have suggested that it is formed from a must-derived precursor, thus its formation depends largely on the formation of its precursor during must processing. It is not greatly influenced by the initial must nitrogen content and source. Interestingly, concentrations of 2-phenyl ethanol in the YPF wines, particularly in the EC1118 samples, were above the aroma threshold which elicits floral and rose odour.

Although the acetic acid ethyl ester formation was different among the treatments, its concentration was below the limit of quantification and had no impact on the aroma of the YPF wines (**Figure 4-53**). Similar to the previous trial, there is probably a correlation between the high formation of acetic acid esters, butanoic acid ethyl ester, fatty acids and their ethyl esters by strain EC1118 with the high VCombi addition (**Figure 4-53** and **Table 4-31**, **4-33**, **4-34**) and its fast fermentation kinetics (**Figure 4-49**). Ethyl esters can be formed from the catabolic activity of alcohol acetyl transferases on ethanol and carboxylic acids (Mason & Dufour, 2000; Lilly et al., 2000, 2006). This nutrient condition might enhance the yeast metabolic activity as well as regulated the metabolic pathways of these ester formations. Moreover, the addition of a high VCombi level, which is DAP plus thiamine, might provide a higher proportion of DAP than another nutrient. When a large pool of DAP and ammonium was added, the amino acids were not used for cellular structures and growth, but rather induced the formation of secondary metabolites like esters (see earlier discussions and

literatures in **5.1.1** and **5.3.2**). In addition, it seemed that the great concentration of MCFA biosynthesis was responsible for the formation of corresponding ethyl esters rather than esterification (Saerens et al., 2006, 2008). On the other hand, the high production of ethyl ester of isobutanoic acid, propionic acid and lactic acid seemed to be related to the sluggish or stuck fermentation of strain VL3 in the OptiWhite treatment followed by strain X5 (**Figure 4-49** and **Table 4-31**, **4-32**). The OptiWhite is an IDY product, which has been reported to release a large pool of amino acids into the juice (Pozo-Bayón et al., 2009 b; Raynal et al., 2009). Amino acids play a direct role as precursors of esters (Hernández-Orte et al., 2005, 2006 b; Miller et al., 2007), thus high formations of ethyl ester in the OptiWhite sample might be observed. In addition, Bowyer et al. (2010) also reported that glutathione, which is rich in OptiWhite, can be used as a natural antioxidant for the preservation of wine aromas and concurrently delay the appearance of developed characters. Thus, it can be suggested that depending on the yeast strain utilized, the formation of acetic acid ester and ethyl esters showed different responses when nutrient/nitrogen was added to the medium as demonstrated in some studies (Torrea et al., 2003, 2005; Miller et al., 2007). Concentrations of acetic acid 3-methylbutyl ester and most ethyl esters in these YPF wines were greatly beyond their perception thresholds, which have a reminiscence of banana, floral and fruity aromas (see reported literatures in **5.1.1**).

Similar to previous YPF trials, the three yeast strains released similar amounts of α-terpineol in all nutrient conditions of the YPF wines (**Figure 4-54**). It might be due to the fact that the activity of β-glycosidase is limited at low pH (pH 5) and the extreme clarification of YPF juice might inhibit its activity as well (see previous literatures in **5.3.3**). In addition, its levels were below the aroma threshold, thus they did not contribute to the varietal aroma (**Table 2-4**).

5.4 Volatile thiols involved in varietal aroma of yellow passion fruit wines

The concentrations of volatile thiols that contribute to the varietal aroma of grape wines may depend considerably on the variety, as well as soil, climate and vineyard management techniques (Rapp, 1998; Tominaga et al., 1998 b; Ribéreau-Gayon et al., 2006 b). Some research works indicated that commercial yeast strains differ in the ability to release various volatile thiols (Murat et al., 2001 a; Howell et al., 2004; Curtin et al., 2009; Swiegers et al., 2009). In addition, the particular yeast's ability to release one thiol does not appear to be linked with its ability to release a second, different volatile thiol. This study also clearly outlines agreeing with the literatures above, that the choice of commercial *Saccharomyces* yeasts had a significant influence, variable according to the specific commercial yeast strain, on the concentration of the typical thiol aromas in the YPF wines.

The volatile thiol analysis is a high-sophisticated analytical method and very time consuming. Therefore, the YPF wines used for the investigation of volatile thiols were chosen from two of four YPF wine trials (**4.3.3** and **4.3.4**). The selection was based on wines, which showed desirable fermentation characteristics and high levels of fermentation-derived compounds that impart pleasant fruity and floral aromas, but very low levels of undesirable compounds.

The results confirmed that high amounts of 3SH and 3SHA can be found as the naturally occurring volatile thiols in YPF juice (Engel & Tressl, 1983, 1991; Weber et al., 1994, 1995; Tominaga & Dubourdieu, 2000). Although the prepared YPF juice in this study was diluted by water addition, these volatile thiols were detected (**Figure 4-55**). In grapes, 3SH is mainly present in conjugate form, while in YPF juice this volatile thiol is present in both free and conjugate form (Tominaga & Dubourdieu, 2000). They also demonstrated that the precursor of 3SH, *S*-(3-hexan-1-ol)-L-cysteine, is present in YPF juice as well. In passion fruit juice, the conjugates are probably converted into free thiols either by acid hydrolysis or by an endogenous enzyme, such as β-lyase.

The results clearly showed that commercial *Saccharomyces* yeasts released approximately 7.1-fold to 11.0-fold of 3SH in YPF wines in comparison to detected 3SH in the YPF juice, however this depended on the yeast strain. According to Tominaga & Dubourdieu (2000), 3SH can be generated by the action of C-S β-lyase from its *S*-cysteine conjugate in YPF juice. As shown in **Figure 4-55**, the high VCombi addition (0.5 g L^{-1}) induced strain EC1118 to be the most effective producer of 3SH and 3SHA in the YPF wines. The LittoLevure strain seemed to follow a similar pattern of 3SH liberation, but less 3SHA production. Strain VL3 appeared to be the least efficient in releasing the 3SH aroma. This result confirmed previous studies in grape wines (Murat et al., 2001 a; Howell et al., 2005; King et al., 2008; Swiegers et al., 2009) that the release and the modulation mechanisms of volatile thiol compounds in YPF wines were also yeast strain dependent.

It has been shown that during fermentation 3SHA is generally formed when acetic acid esterifies the 3SH that has been released. Swiegers et al. (2005 b, 2007) found out that 3SHA is formed from 3SH by the action of the yeast ester-forming alcohol acetyltransferase, encoded by the *ATF1* gene. Lilly et al. (2006) suggested that the overexpression of *ATF1* in a VIN13 yeast strain resulted in increased 3SHA concentrations. The present study also revealed that a higher concentration of 3SH induced an increased production of 3SHA in YPF wines for most yeast strains (6.5 to 9.0 % released 3SHA), except for strain LittoLevure (5.0 %). Some specific gene, which encodes an ester-degrading enzyme, might be involved (Swiegers et al., 2005 b, 2007).

Interestingly, concentrations of 3SH in YPF wines were between 59-fold and 93-fold (in the EC1118 treatment) of the perception thresholds (60 ng L^{-1}), which contribute to grapefruit and passion fruit aromas (Tominaga et al., 1998 b; Bell & Henschke, 2005; Francis & Newton, 2005; Dubourdieu et al., 2006; Ribéreau-Gayon et al., 2006 b). Concentrations of 3SHA (boxwood, grape fruit zest, passion fruit aromas) in YPF wines were also above the aroma threshold (4 ng L^{-1}) and differed from 57-fold to 125-fold (in the EC1118 treatment).

To examine the effect of the nutrient source (DAP and VCombi) at different levels (0.25 and 0.5 g L^{-1}) on the production of volatile thiols, the two yeast strains of EC1118 and X5 were used. As demonstrated in **Figure 4-56**, the result clearly indicated that the low level of DAP addition modulated higher 3SH and 3SHA productions by strain EC1118 than the high level. This result confirmed the investigation of Subileau et al. (2008) that a complementation with DAP induces a decrease of 3SH production. They also concluded that in synthetic medium, Cys-3SH enters the cell through at least one identified transporter, GAP1p, whose activity is limiting the release of volatile thiols. The uptake of the precursor through GAP1p is not confirmed, but the effect of the addition of DAP, eventually prolonging nitrogen catabolite repression, is shown to decrease thiol production. On the other hand, the addition of VCombi (DAP plus thiamine) at high level tended to enhance higher expression of these thiols in strain EC1118. In addition, the nutrient source and concentration had no significant influence on the 3SH release in strain X5.

As discussed above, 3SHA is formed from 3SH by the action of the yeast activity (Swiegers et al., 2005 b, 2007), thus higher concentration of 3SH seemed to determine greater production of 3SHA in the YPF wines. For instance, the EC1118 strain with the addition of low DAP level and high VCombi level resulted in the highest transformation of 3SHA from 3SH at an average of 5.7 % and 9.0 % respectively. In addition, concentrations of both 3SH and 3SHA were quite beyond the aroma threshold, which contributes to the varietal aroma (see literatures above and **Table 2-5**).

As shown in **Figure 4-57**, commercial *Saccharomyces* yeast strains significantly had the greater impact on the liberation of 3SH in YPF wines than the nutrient source supplemented in the YPF juices. The X5 strain liberated higher concentrations of 3SH in the YPF wine in either the VCombi or VUltra supplementation (5527.5 $\pm$ 639.5 and 5206.1 $\pm$ 397.1 ng L^{-1}, respectively) than strain EC1118. The results showed that neither the yeast strain nor the nutrient source affected the 3SHA formation ranging from 263.8 $\pm$ 73.5 to 333.9 $\pm$ 47.5 ng L^{-1}, although the 3SHA amounts tended a little bit to be higher in the VCombi treatment for strain EC1118. In contrast to the results described above, the addition of VCombi stimulated the EC1118 strain to release higher 3SH amounts than strain X5. It is important to take into account that these results were obtained from the lower nutrient level addition (0.4 g L^{-1}

VCombi) hence it might influence the ability of the yeast strain in the liberation of volatile thiols in final YPF wines.

Subileau et al. (2008) recently investigated that a complementation with DAP in grape must induces a decrease of 3SH production. On the other hand, the addition of DAP, in form of VCombi and VUltra, seemed to have no impact on the release of 3SH during YPF juice fermentation. It has been suggested by some studies that the particular yeast's ability to release one thiol does not appear to be linked with its ability to release a second, different thiol (Murat et al., 2001 a; Swiegers et al., 2005 b, 2009). In addition, separate yeast enzymes may be involved in the formation of different volatile thiols, allowing the levels of the aroma compounds to be altered independently (Murat et al., 2001 a; Dubourdieu et al., 2006). The genetic and physiological characteristics of each commercial yeast strain might be involved (Murat et al., 2001 a; Howell et al., 2005; Curtin et al., 2009; Swiegers et al., 2009).

Concentrations of 3SH and 3SHA in these YPF wines were far beyond the perception thresholds as shown in publications related to grape wines (see earlier literatures above and **Table 2-5**) at about 69-fold to 92-fold and 66-fold to 83-fold, respectively.

The present result clearly confirmed that the presence of larger quantities of varietal volatile thiols in YPF wines than in YPF juice is due to the metabolic action of the commercial *Saccharomyces* yeasts. Tominaga & Dubourdieu (2000) also suggested that the conjugates in YPF juice are probably converted into free thiols either by acid hydrolysis or by an endogenous enzyme like β-lyase. Furthermore, the qualitative and quantitative composition of thiol-precursors can affect the concentration of thiols in final wines.

The structures of 3SH, 3SHA and their cysteinylated precursors in YPF juices have been identified (Engel & Tressl, 1983, 1991; Weber et al., 1994, 1995; Tominaga & Dubourdieu, 2000). A little scientific research carried out the impact of yeast rehydration nutrients such as DYNASTART® on the increased release of volatile thiols by yeasts in Sauvignon blanc wine (Bowyer et al., 2008; Swiegers et al., 2008; van der Westhuizen et al., 2008). However, the impact of yeast strains and nitrogen supplementation on the release of volatile varietal thiols in YPF wine has not yet been elucidated. Therefore, this study is the first to report the effect of commercial *Saccharomyces* yeast strains and nutrient supplementations on volatile thiol production in YPF wines.

6. CONCLUSION AND PERSPECTIVES

Yeast fermentation of sugars not only produces ethanol and carbon dioxide but also a range of minor but sensorially important volatile metabolites which give the wine the vinous character and inhibit the development of off-flavours. These volatile metabolites, which comprise esters, higher alcohols, carbonyls, volatile fatty acids and sulphur compounds, are derived from sugar and amino acid metabolism. Hence, the assessment of nitrogen requirements for wine yeasts should be controlled because it can not only have an impact on yeast growth and fermentation kinetics but also on the formation of volatile and non-volatile compounds that are important for the organoleptic qualities of the resulting wine. The limitation of nitrogen and its sources/composition can also influence the formation of reduced sulphur compounds like H_2S.

One of the main objectives in the selection of industrial yeasts for the beverage industry must be the understanding of the relationship between the nutrient supplements and the production of desirable non-volatile and volatile compounds by different commercial yeast strains. Therefore, the main objective of this study is focused on the improvement of desirable metabolites, the varietal and important aromas in grape wines from two varieties of Sauvignon blanc and Scheurebe as well as in yellow passion fruit wine by the optimal choice of *Saccharomyces* yeast strains and nutrient supplements. In addition, the fermentation trials of passion fruit wines were carried out accordingly on the basis of the experimental results obtained from the research on grape wines.

The optimal choice of the required yeast strains and nutrient supplements for the fermentation of grape wines and YPF wines were studied in detail. The grape juices and YPF juices were fermented at 20 ^{0}C under controlled conditions. The growth and fermentative behaviours, volatile compound productions and other enological characteristics, which have an impact on grape wine and YPF wine properties, were summarized as below.

The fermentation trials of Scheurebe wine:

It was observed that the supplemented nutrient sources (control/without nutrient, OptiWhite, Superstart, DAP and Fermaid E) in the fermentations of fresh Scheurebe juice strongly modulated the wine composition in a strain-dependent manner (EC1118 and X5). For some compounds, the influence of the nitrogen source is relatively similar for the two strains, whereas for the other compounds, considerable differences were observed. Nevertheless, Fermaid E blanc followed by DAP and Superstart seemed to be the effective nutrients to accelerate fermentation kinetics and to promote high formations of important wine aromas, but to diminish the formation of SO_2-binding compounds for the two strains.

The impact of thirteen commercial strains of *Saccharomyces* yeasts (EC1118, Sauvignon, VL3, X5, X16, VIN13, Alchemy I, Alchemy II, 4F9, LVCB, LittoLevure, AWRI R2 and QA23) without nutrient addition was evaluated on fermentation characteristics and on the production of metabolic compounds of obtained Scheurebe wines. The results clearly indicate some differences among the yeast strains used. The VIN13, AWRI R2, EC1118, QA23, VL3 and X16 strains produced wines with higher volatile contents; mainly 2-phenyl ethanol, acetic acid 2-phenyl ethyl ester, linalool and wine esters and less negative and undesirable compounds. It was realized that strain QA23 has to be well observed as it had the slowest fermentation, although it completely finished fermentation with low residual sugar.

In conclusion, on the basis of the results obtained with regard to fermentation characteristics as well as important and desirable aromas, the optimal choice of commercial *Saccharomyces* yeast strains for the fermentation of Scheurebe wine are strains VIN13, AWRI R2, EC1118, QA23, VL3, X5 and X16. The most effective nutrients are Fermaid E blanc, DAP and Superstart at 0.3 g L^{-1}.

The fermentation trials of Sauvignon blanc wine:

The effect of the nutrient source (Fermaid E and OptiWhite at 0.3 g L^{-1}) on the fermentation kinetics, non-volatile and volatile compounds of the wines was dependent upon the yeast strain used (EC1118, Sauvignon, VL3, X5, VIN7, VIN13, 4F9, AWRI R2 and LVCB). The results indicated that Fermaid E addition increased the formation of ethyl esters and acetic acid esters, but significantly decreased the formation of keto acids for most yeast strains. It is worth noting that the nine yeast strains showed similar formations of acetaldehyde, higher alcohols, most ethyl esters and α-terpineol with less response to the nutrient source. Nonetheless, strains EC1118, Sauvignon, VL3, X5 and VIN7 were the most optimal yeast strains, thus their behaviour was intensively investigated in further trials.

The fermentation trial with five yeast strains (EC1118, X5, VIN7, Alchemy I and LittoLevure) showed that some significant differences with regard to fermentation kinetics, non-volatile and volatile metabolic compounds in the wines were depended on the nutrient sources (Superstart, Fermaid O, Fermaid E and VUltra). The Fermaid E and VUltra seemed to be the most effective nutrients to enhance the fermentation behaviours and the formation of desirable metabolic compounds in strains EC1118, X5 and Alchemy I with the lower level of volatile acidity and keto acids.

Finally, the outcomes clearly indicated the direct influence of commercial yeast strains and nutrient supplements on the different fermentation parameters and the formation of important wine aromas. Under laboratory conditions, yeast strains X5, EC1118, Alchemy I and II and VL3 and/or the nutrient sources of Fermaid E blanc, DAP and Superstart at high levels seemed to be the most effective for the fermentation of Sauvignon blanc wine.

The fermentation trials of yellow passion fruit (YPF) wine:

The following trials investigated the improvement of fermentation properties, important non-volatile and volatile compounds as well as varietal aromas in the YPF wines by the optimal choice of commercial yeast strains and nutrients. This choice was leaned on the results obtained from the trials carried out with Scheurebe and Sauvignon blanc wines.
The optimal fermentation parameters and the formation of metabolic compounds in the YPF wines were evaluated for five *S. cerevisiae* var. *bayanus* strains (EC1118, AWRI R2, LittoLevure, QA23 and Freddo), seven *S. cerevisiae* strains (Sauvignon, VL3, X5, X16, 4F9, VIN13 and LVCB) and two *Saccharomyces spp.* strains (Alchemy I and II) with the same nutrient addition (0.5 g L^{-1} DAP). The results clearly showed that strains QA23 and LittoLevure were the most optimal yeast strains for the production of YPF wines displaying more desirable aroma compounds, particularly 2-phenyl ethanol, acetic acid 2-phenyl ethyl ester, most of wine esters and linalool but a low level of keto acids and reductive sulphur compounds, followed by strains EC1118, X5, VL3, Alchemy I and II. These optimal yeast strains were also used for an intensive investigation and a better understanding in further trials of YPF wines.

At the mean time, the influence of three yeast strains (EC1118, Sauvignon and X5) and five nutrient sources (DAP, VCombi, VUltra, Fermaid E and OptiWhite at 0.4 g L^{-1}) on the fermentation behaviour and the formation of non-volatile and volatile compounds as well as monoterpenes was also examined. The results demonstrated that supplementation of YPF juices with VCombi, VUltra or Fermaid E appeared to be the most effective method to stimulate the production of great amounts of important and desirable volatile components, mainly wine esters, 2-phenyl ethanol and its ester, but low levels of keto acids for the three yeast strains, nevertheless this was slightly yeast strain-dependent. Interestingly, strain Sauvignon also released great amounts of linalool and α-terpineol in the YPF wines.

The effects of yeast strains (EC1118, VL3, X5, Alchemy I and LittoLevure), nutrient sources (DAP and VCombi) and nutrient levels (0.25 and 0.5 g L^{-1}) were examined to improve the fermentation characteristics, wine aroma compounds as well as varietal volatile thiols in the YPF wines. The results clearly demonstrated that supplementation of YPF juice with VCombi (DAP plus thiamine) at a high level appeared to be the most effective way of producing YPF wines with accelerated fermentation kinetics, more desirable aromas and a low level of reductive sulphur compounds and keto acids for strains EC1118, VL3, X5 and LittoLevure. Interestingly, strain EC1118 was the greatest producer of varietal volatile thiols, 3SH and 3SHA, in 0.25 g L^{-1} DAP and 0.5 g L^{-1} VCombi treatments. Strain LittoLevure with 0.5 g L^{-1} VCombi addition also produced high amounts of 3SHA.

The final YPF wine fermentations with three yeast strains (EC1118, VL3 and X5) showed some significant differences with regard to the fermentation parameters, non-volatile and volatile compounds and volatile thiols depending on different nutrient supplements (DAP, VCombi, VUltra, Fermaid E and OptiWhite) at two levels (0.2 and 0.4 g L^{-1}). The results revealed that the addition of VCombi and VUltra at 0.4 g L^{-1} resulted in enhanced fermentation behaviours and a high level of desirable aroma compounds, especially 2-phenyl ethanol, acetic acid 2-phenyl ethyl ester, but a lower level of higher alcohols, keto acids for the three yeast strains. In addition, the X5 strain with the addition of high VCombi or VUltra produced the YPF wine having the greatest concentration of 3SH, while concentrations of 3SHA were similar among the yeast strains and nutrients used.

Finally, the YPF results obtained from YPF fermentations revealed that when 0.5 g L^{-1} DAP was added as sole source of nitrogen in YPF juice, strains QA23, LittoLevure, EC1118, X5, VL3 and Alchemy I and II seemed to be the optimal choice for the YPF wine fermentation. However, the addition of VCombi at high levels (0.5 g L^{-1}) was the most efficient nutrient condition to obtain the best fermentation characteristics and to improve wine aroma and varietal volatile thiols in the YPF wines for strains EC1118, LittoLevure, X5, Alchemy I and VL3, respectively. The addition of either 0.25 g L^{-1} DAP or 0.4-0.5 g L^{-1} VCombi and VUltra modulated varietal volatile thiol productions in the YPF wines by strains EC1118 and strain X5 as well. Nevertheless, it has to be taken into account that DAP addition resulted in excessive formation of keto acids by strain X5. Interestingly, concentrations of volatile thiols in YPF wines were quite above the aroma threshold contributing to typical and varietal aromas of grapefruit zest and passion fruit.

In conclusion, this present study clearly indicated that the use of different commercial *Saccharomyces* strains for grape wine and YPF wine fermentations resulted in wines and YPF wines with differing fermentative characteristics and the production of non-volatile and volatile compounds that influenced the quality. The nutrients supplemented to the fermentations strongly modulated the wine composition in a strain-dependent manner as well. This study is particularly relevant considering that in grape wine and tropical fruit wine productions, it is simultaneously desirable to achieve low concentrations of SO_2-binding compounds, high levels of substances that impart pleasant fruity and floral aromas as well as varietal aromas. On the other hand, low levels of compounds that can cause off-flavours and low levels of metabolites (e.g. MCFAs) that could compromise the yeast fermentative activity have to be achieved.

Therefore, the present work is demonstrating that the choice of the yeast strain as well as the nutrient support can have a high impact not only on the fermentation activity, but also on the composition of volatile and non-volatile compounds. The results may help the winemakers to optimize the important and potential fermentation parameters by an adequate choice of yeast strains and nutrient supplements to improve wine quality.
In addition, this study also has important implications for the grape and tropical fruit winemaking industry where a better understanding of the nutritional requirements of *Saccharomyces* is necessary to reduce fermentation problems and to improve the final product quality. It is worthwhile pointing out that this work is an interesting new observation for the YPF wine fermentation, although the fermentation trials were only done in laboratory scale. Thus, some different fermentation parameters as well as volatile compounds might be achieved under industrial conditions.

Nevertheless, a better understanding of the effects of nutrient supplementation on yeast metabolic products as well as sensory properties is still required in further work in both the laboratory and pilot scale.

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8. ADDENDUM

Additional results which are described in this chapter are related to Scheurebe and Sauvignon blanc grape juices and YPF juices which were used in different research trials of this study.

In **Table A-1, A-2** and **A-3** the amino acid concentrations which were detected in the diverse juices are listed.

Table A-1 Concentration of amino acids in Scheurebe grape juice used in trials described in chapter **3.7.1** to **3.7.2**

Amino acid (mg L^{-1})	Juice 2007	Juice 2008
Aspartic acid	70.2	62.3
Threonine	65.7	77.6
Serine	38.7	51.5
Asparagine	8.2	9.2
Glutamic acid	79.2	98.2
Glutamine	174.8	173.4
α-Aminoadipic acid	n.d.	n.d.
Glycine	2.0	2.8
Alanine	96.2	92.9
Citrulline	6.0	10.5
α-Aminobutyric acid	n.d.	n.d.
Valine	16.7	29.4
Cystine	n.d.	n.d.
Methionine	2.7	8.0
Isoleucine	12.7	27.1
Leucine	13.4	33.3
Tyrosine	5.6	8.9
Phenylalanine	19.4	41.0
β-Alanine	2.0	2.5
β -Aminobutyric acid	n.d.	n.d.
γ-Aminobutyric acid	58.8	60.8
Histidine	11.2	17.6
Tryptophan	4.0	8.7
Ornithine	1.1	1.4
Lysine	3.1	5.7
Ammonia	54.9	66.4
Arginine	299.7	425.3
Proline	256.2	169.4
Total amino acids	1302.5	1484.0
Total amino acids (without proline)	1046.3	1314.6

n.d.: not detectable

Table A-2 Concentration of amino acids in fresh Sauvignon blanc grape juice used in trials described in chapter **3.8.1** to **3.8.2**

Amino acid (mg L^{-1})	Sauvignon blanc juice 2008	Sauvignon blanc juice 2009
Aspartic acid	70.5	64.0
Threonine	81.0	90.7
Serine	47.2	69.8
Asparagine	3.5	4.8
Glutamic acid	82.7	158.6
Glutamine	99.0	128.7
α-Aminoadipic acid	n.d.	n.d.
Glycine	1.9	3.0
Alanine	138.8	209.7
Citrulline	11.9	15.1
α-Aminobutyric acid	0.4	0.4
Valine	10.7	20.1
Cystine	n.d.	n.d.
Methionine	1.4	1.5
Isoleucine	7.8	10.3
Leucine	10.2	15.4
Tyrosine	3.5	6.4
Phenylalanine	18.4	18.9
β-Alanine	1.3	3.5
γ-Aminobutyric acid	43.6	90.1
Histidine	10.8	23.3
Tryptophan	1.8	2.2
Ornithine	1.8	2.8
Lysine	2.2	1.8
Ammonia	69.9	70.2
Arginine	544.0	771.8
Proline	115.4	430.8
Total amino acids	1380.0	2213.7
Total amino acids (without proline)	1264.6	1782.9

n.d.: not detectable

Table A-3 Concentration of amino acids in prepared YPF juice used in YPF trials described in chapter **3.9.1** to **3.9.4**

Amino acids (mg L^{-1})	YPF puree	Lists of prepared YPF juices			
		1st trial [a]	2nd trial [b]	3rd trial [c]	4th trial [d]
Aspartic acid	569.3	55.5	47.6	32.0	43.6
Threonine	13.8	1.1	1.0	0.7	1.0
Serine	201.3	19.1	16.9	11.1	15.9
Asparagine	6.1	n.d.	n.d.	n.d.	n.d.
Glutamic acid	709.8	64.1	54.7	37.7	49.3
Glutamine	82.7	7.2	7.0	4.2	5.6
α-Aminoadipic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Glycine	7.1	0.7	0.7	0.7	0.8
Alanine	151.7	14.5	12.6	8.7	11.5
Citrulline	n.d.	n.d.	n.d.	n.d.	n.d.
α-Aminobutyric acid	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	32.7	2.7	2.4	1.6	2.2
Cystine	n.d.	n.d.	n.d.	n.d.	n.d.
Methionine	4.0	n.d.	n.d.	n.d.	n.d.
Isoleucine	17.0	1.6	1.4	1.3	1.3
Leucine	30.7	2.9	2.5	1.9	2.3
Tyrosine	16.4	1.3	1.6	1.6	1.3
Phenylalanine	46.5	4.1	3.6	2.1	3.5
β-Alanine	8.0	0.6	0.6	0.3	0.7
γ-Aminobutyric acid	172.1	16.9	14.9	10.1	13.3
Histidine	23.5	2.2	1.9	1.3	1.9
Tryptophan	n.d.	n.d.	n.d.	n.d.	n.d.
Ornithine	0.4	n.d.	n.d.	n.d.	n.d.
Lysine	29.5	2.3	2.0	1.3	1.8
Ammonia	56.9	6.2	5.4	3.9	5.1
Arginine	47.0	4.1	3.9	2.4	3.0
Proline	153.1	12.5	11.2	8.4	11.1
Total amino acids	2644.1	219.6	191.5	131.6	175.6
Total amino acids (without proline)	2491.0	207.1	180.3	123.1	164.4

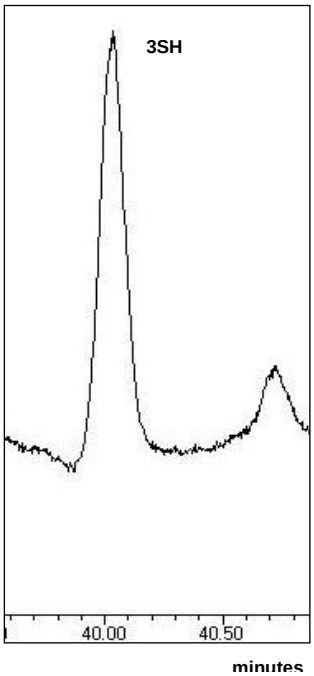
n.d.: not detectable

[a] amino acid composition in prepared YPF juice for the 1st YPF trial described in chapter **3.9.1**

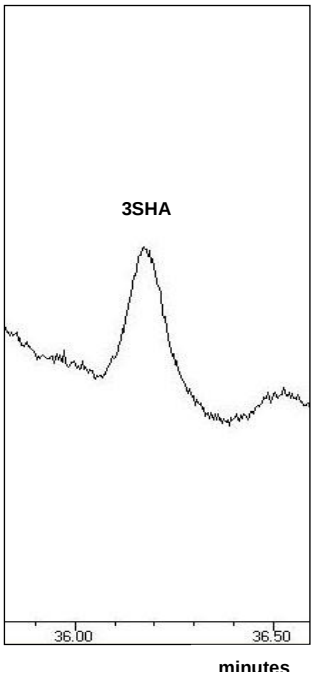
[b] amino acid composition in prepared YPF juice for the 2nd YPF trial described in chapter **3.9.2**

[c] amino acid composition in prepared YPF juice for the 3rd YPF trial described in chapter **3.9.3**

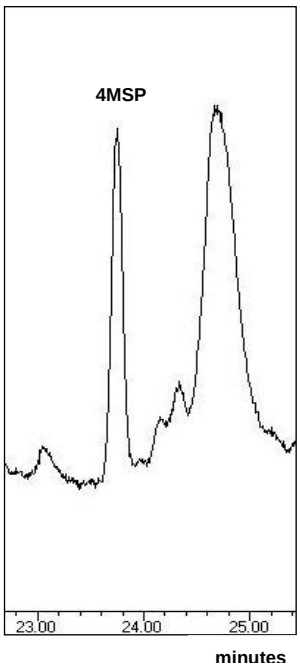
[d] amino acid composition in prepared YPF juice for the 4th YPF trial described in chapter **3.9.4**



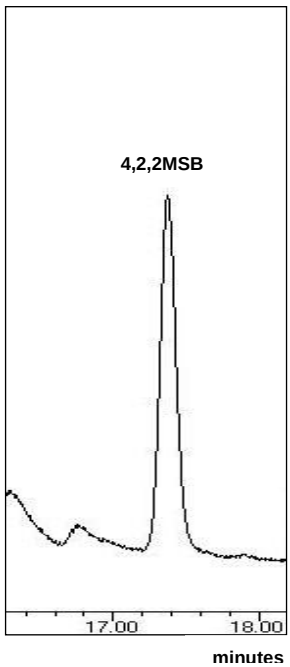
a) 3-Sulphanylhexanol (m/z 134) 1000 ng L^{-1*}



b) Acetic acid 3-sulphanylhexyl ester (m/z 116) 100 ng L^{-1*}



c) 4-Methyl-4-sulphanylpentan-2-one (m/z 132) 40 ng L^{-1*}



d) 4-Methoxy-2-methyl-2-sulphanylbutane (m/z 100) 200 ng L^{-1*} (Internal standard)

Figure A-1 Retention time windows of selected ion chromatograms showing the quantifier ion peaks of the four thiols obtained in the analysis of the dearomatized wine sample spiked with different levels of the analytes

*concentration added to dearomatized wine

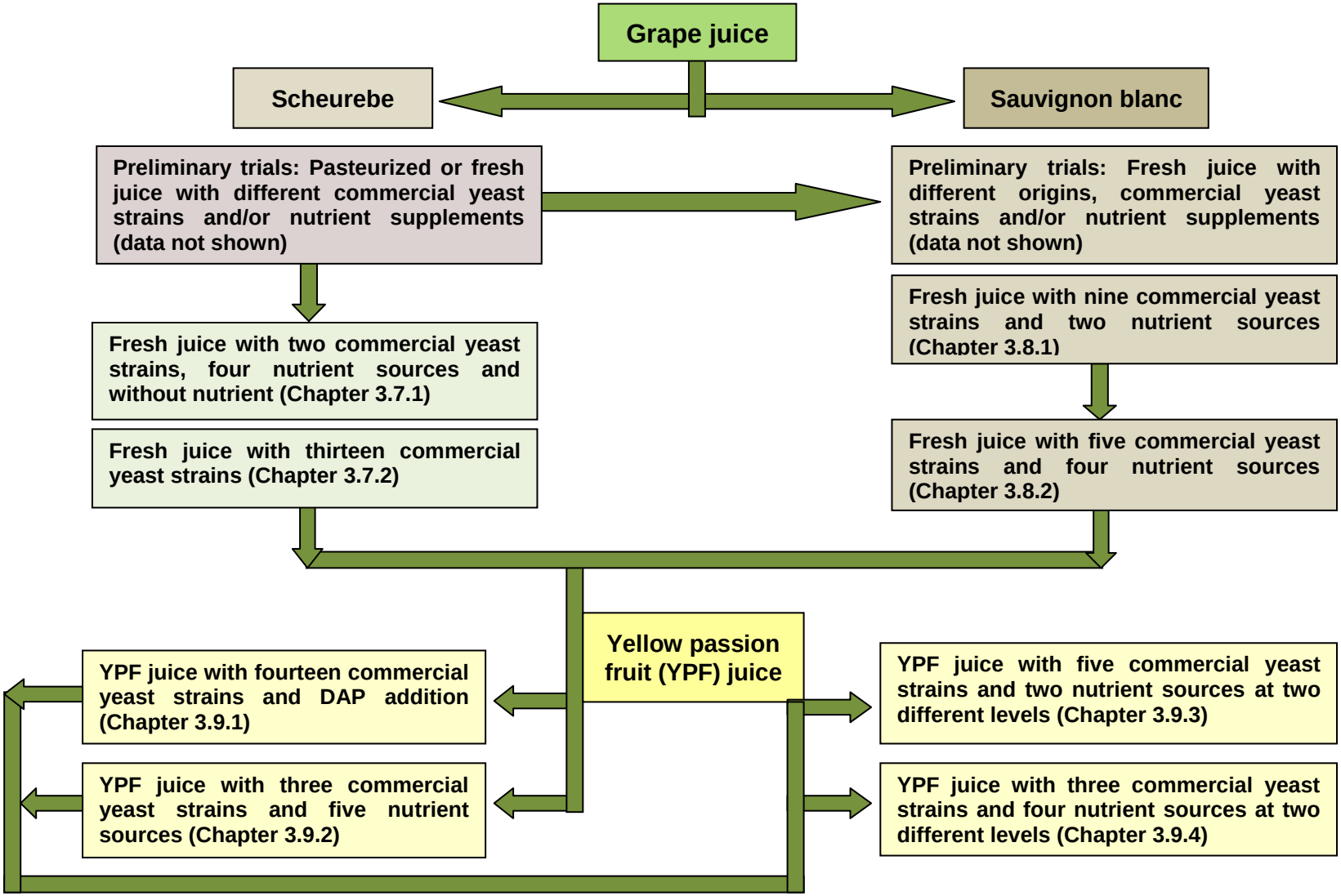


Figure A-2 Experimental outline for the fermentation trials of Scheurebe and Sauvignon blanc grape juices and YPF juices

List of publication and presentation

Publication:

Srisamatthakarn, P., Rauhut, D., & Brückner, H. (2010). Impact of commercial yeast strains on wine fermentation and formation of metabolites of yellow passion fruit juice (*Passiflora edulis* Sims f. *flavicarpa* Degner). *Asian Journal of Food and Agro-Industry*, *3* (2), 282-292.

Poster:

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Erklärung

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